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(57) Abstract

Cyclic peptides and compositions comprising such cyclic peptides are provided. The cyclic peptides comprise a cadherin cell adhesion recognition sequence HAV. Methods for using such peptides and compositions for modulating synaptic stability are also provided.

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COMPOUNDS AND METHODS FOR MODULATING SYNAPTIC STABILITY

TECHNICAL FIELD

The present invention relates generally to methods for modulating cell adhesion, and more particularly to cyclic peptides comprising a cadherin cell adhesion recognition sequence, and to the use of such cyclic peptides for inhibiting or enhancing cadherin-mediated cell adhesion.

BACKGROUND OF THE INVENTION

Cell adhesion is a complex process that is important for maintaining tissue integrity and generating physical and permeability barriers within the body. All tissues are divided into discrete compartments, each of which is composed of a specific cell type that adheres to similar cell types. Such adhesion triggers the formation of intercellular junctions (*i.e.*, readily definable contact sites on the surfaces of adjacent cells that are adhering to one another), also known as tight junctions, gap junctions and belt desmosomes. The formation of such junctions gives rise to physical and permeability barriers that restrict the free passage of cells and other biological substances from one tissue compartment to another. For example, the blood vessels of all tissues are composed of endothelial cells. In order for components in the blood to enter a given tissue compartment, they must first pass from the lumen of a blood vessel through the barrier formed by the endothelial cells of that vessel. Similarly, in order for substances to enter the body via the gut, the substances must first pass through a barrier formed by the epithelial cells of that tissue. To enter the blood via the skin, both epithelial and endothelial cell layers must be crossed.

Cell adhesion is mediated by specific cell surface adhesion molecules (CAMs). There are many different families of CAMs, including the immunoglobulin, integrin, selectin and cadherin superfamilies, and each cell type expresses a unique

combination of these molecules. Cadherins are a rapidly expanding family of calcium-dependent CAMs (Munro et al., *In: Cell Adhesion and Invasion in Cancer Metastasis,* P. Brodt, ed., pp. 17-34, RG Landes Co.(Austin TX, 1996). The classical cadherins (abbreviated CADs) are integral membrane glycoproteins that generally promote cell adhesion through homophilic interactions (a CAD on the surface of one cell binds to an identical CAD on the surface of another cell), although CADs also appear to be capable of forming heterotypic complexes with one another under certain circumstances and with lower affinity. Cadherins have been shown to regulate epithelial, endothelial, neural and cancer cell adhesion, with different CADs expressed on different cell types. N (neural) - cadherin is predominantly expressed by neural cells, endothelial cells and a variety of cancer cell types. E (epithelial) - cadherin is predominantly expressed by epithelial cells. Other CADs are P (placental) - cadherin, which is found in human skin and R (retinal) - cadherin. A detailed discussion of the classical cadherins is provided in Munro SB et al., 1996, *In: Cell Adhesion and Invasion in Cancer Metastasis,* P. Brodt, ed., pp.17-34 (RG Landes Company, Austin TX).

The structures of the CADs are generally similar. As illustrated in Figure 1, CADs are composed of five extracellular domains (EC1-EC5), a single hydrophobic domain (TM) that transverses the plasma membrane (PM), and two cytoplasmic domains (CP1 and CP2). The calcium binding motifs DXNDN (SEQ ID NO:41), DXD and LDRE (SEQ ID NO:40) are interspersed throughout the extracellular domains. The first extracellular domain (EC1) contains the classical cadherin cell adhesion recognition (CAR) sequence, HAV (His-Ala-Val), along with flanking sequences on either side of the CAR sequence that may play a role in conferring specificity. Synthetic peptides containing the CAR sequence and antibodies directed against the CAR sequence have been shown to inhibit CAD-dependent processes (Munro et al., *supra*; Blaschuk et al., *J. Mol. Biol. 211*:679-82, 1990; Blaschuk et al., *Develop. Biol. 139*:227-29, 1990; Alexander et al., *J. Cell. Physiol. 156*:610-18, 1993). The three-dimensional solution and crystal structures of the EC1 domain have been determined (Overduin et al., *Science 267*:386-389, 1995; Shapiro et al., *Nature 374*:327-337, 1995).

Although cell adhesion is required for certain normal physiological functions, there are situations in which cell adhesion is undesirable. For example, many pathologies (such as autoimmune and inflammatory diseases) involve abnormal cellular adhesion. In addition, cadherin-mediated cell adhesion plays an important role in mediating synaptic plasticity, which is necessary for repair processes within the brain, as well as learning and memory. Modulation of cell adhesion may be desirable in certain circumstances, but there are currently no effective methods for modulating synaptic plasticity in a mammal.

Accordingly, there is a need in the art for compounds that modulate cell adhesion and improve synaptic stability. The present invention fulfills this need and further provides other related advantages.

SUMMARY OF THE INVENTION

The present invention provides cyclic peptides and methods for modulating cadherin-mediated cell adhesion. Within one aspect, the present invention provides cyclic peptides comprising the sequence His-Ala-Val, wherein the cyclic peptides modulate cadherin-mediated cell adhesion. Within one embodiment a cyclic peptide has the formula:

$$(Z_1)\text{-}(Y_1)\text{-}(X_1)\text{-His-Ala-Val-}(X_2)\text{-}(Y_2)\text{-}(Z_2)$$

wherein X_1, and X_2 are optional, and if present, are independently selected from the group consisting of amino acid residues and combinations thereof in which the residues are linked by peptide bonds, and wherein X_1 and X_2 independently range in size from 0 to 10 residues, such that the sum of residues contained within X_1 and X_2 ranges from 1 to 12; wherein Y_1 and Y_2 are independently selected from the group consisting of amino acid residues, and wherein a covalent bond is formed between residues Y_1 and Y_2; and wherein Z_1 and Z_2 are optional, and if present, are independently selected from the group consisting of amino acid residues and combinations thereof in which the residues are linked by peptide bonds. Such cyclic peptides may comprise modifications such as an N-acetyl or N-alkoxybenzyl group

and/or a C-terminal amide or ester group. Cyclic peptides may be cyclized via, for example. a disulfide bond; an amide bond between terminal functional groups, between residue side-chains or between one terminal functional group and one residue side chain; a thioether bond or $\delta_1\delta_1$-ditryptophan, or a derivative thereof.

Within further aspects, the present invention provides cell adhesion modulating agents that comprise a cyclic peptide as described above. Within specific embodiments, such modulating agents may be linked to one or more of a targeting agent, a drug, a solid support or support molecule, or a detectable marker. In addition, or alternatively, a cell adhesion modulating agent may further comprising one or more of: (a) a cell adhesion recognition sequence that is bound by an adhesion molecule other than a cadherin, wherein said cell adhesion recognition sequence is separated from any HAV sequence(s) by a linker; and/or (b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than a cadherin.

The present invention further provides pharmaceutical compositions comprising a cell adhesion modulating agent as described above, in combination with a pharmaceutically acceptable carrier. Such compositions may further comprise a drug. Alternatively, or in addition, such compositions may comprise: (a) a peptide comprising a cell adhesion recognition sequence that is bound by an adhesion molecule other than a cadherin; and/or (b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than a cadherin.

Within further aspects, the present invention provides methods for inhibiting synaptic stability in a mammal, comprising administering to a mammal a cell adhesion modulating agent as described above, wherein the modulating agent inhibits cadherin-mediated cell adhesion.

These and other aspects of the invention will become evident upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each were individually noted for incorporation.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram depicting the structure of classical CADs. The five extracellular domains are designated EC1-EC5, the hydrophobic domain that transverses the plasma membrane (PM) is represented by TM, and the two cytoplasmic domains are represented by CP1 and CP2. The calcium binding motifs are shown by DXNDN (SEQ ID NO:41), DXD and LDRE (SEQ ID NO:40). The CAR sequence, HAV, is shown within EC1. Cytoplasmic proteins β-catenin (β), α-catenin (α) and α-actinin (ACT), which mediate the interaction between CADs and microfilaments (MF) are also shown.

Figure 2 provides the amino acid sequences of mammalian classical cadherin EC1 domains: human N-cadherin (SEQ ID NO:1), mouse N-cadherin (SEQ ID NO:2), cow N-cadherin (SEQ ID NO:3), human P-cadherin (SEQ ID NO:4), mouse P-cadherin (SEQ ID NO:5), human E-cadherin (SEQ ID NO:6) and mouse E-cadherin (SEQ ID NO:7).

Figure 3 provides the structures of representative cyclic peptides of the present invention (structures on the left hand side), along with similar, but inactive, structures (on the right).

Figure 4 is a histogram depicting the mean neurite length in microns for neurons grown on a monolayer of untransfected 3T3 cells (first column) or 3T3 cells transfected with cDNA encoding N-cadherin (columns 2-4). In the third column, the mean neurite length in the presence of the representative cyclic peptide N-Ac-<u>CHAVC</u>-NH_2 (SEQ ID NO:8) is shown. Column 4 depicts the mean neurite length in the presence of the control peptide N-Ac-<u>CHGVC</u>-NH_2 (SEQ ID NO:9).

Figure 5 is a graph showing a dose response curve for the representative cyclic peptide N-Ac-<u>CHAVC</u>-NH_2 (SEQ ID NO:8) on control 3T3 cells (open circles) and on 3T3 cells expressing N-cadherin (solid circles).

Figure 6 is a histogram depicting the mean neurite length in microns for neurons grown in the presence (solid bars) or absence (cross-hatched bars) of 500 μg/mL of the representative cyclic peptide N-Ac-<u>CHAVC</u>-NH_2 (SEQ ID NO:8). In the

first pair of bars, neurons were grown on a monolayer of untransfected 3T3 cells. In the remaining columns, the mean neurite length is shown for neurons cultured on 3T3 cells transfected with cDNA encoding N-CAM (second pair of bars), L1 (third pair of bars) or N-cadherin (fourth pair of bars).

Figures 7A-C are photographs showing monolayer cultures of bovine endothelial cells in the presence (Figure 7A) and absence (Figure 7C) of a representative cyclic peptide or in the presence of an inactive control peptide (Figure 7B). Figure 7A shows the cells 30 minutes after exposure to 500 µg/mL N-Ac-<u>CHAVC</u>-NH_2 (SEQ ID NO:8). Figure 7B shows the cells 30 minutes after exposure to the control peptide N-Ac-<u>CHGVC</u>-NH_2 (SEQ ID NO:9). Figure 7C shows the cells in the absence of cyclic peptide. Note that the endothelial cells retracted from one another in the presence of N-Ac-<u>CHAVC</u>-NH_2 (SEQ ID NO:8).

Figures 8A-C are photographs showing monolayer cultures of bovine endothelial cells in the presence (Figure 8A) and absence (Figure 8C) of a representative cyclic peptide or in the presence of an inactive control peptide (Figure 8B). Figure 8A shows the cells 30 minutes after exposure to 500 µg/mL N-Ac-<u>CAHAVDIC</u>-NH_2 (SEQ ID NO:10). Figure 8B shows the cells 30 minutes after exposure to the control peptide N-Ac-<u>CAHGVDIC</u>-NH_2 (SEQ ID NO:11). Figure 8C shows the cells in the absence of cyclic peptide. In this case, neither of the cyclic peptides show activity.

Figures 9A-C are photographs showing monolayer cultures of bovine endothelial cells in the presence (Figure 9A) and absence (Figure 9C) of a representative cyclic peptide or in the presence of an inactive control peptide (Figure 9B). Figure 9A shows the cells 30 minutes after exposure to 500 µg/mL N-Ac-<u>CAHAVDC</u>-NH_2 (SEQ ID NO:16). Figure 9B shows the cells 30 minutes after exposure to the control peptide N-Ac-<u>CAHGVDC</u>-NH_2 (SEQ ID NO:17). Figure 9C shows the cells in the absence of cyclic peptide. Note that the endothelial cells retracted from one another in the presence of N-Ac-<u>CAHAVDC</u>-NH_2 (SEQ ID NO:16).

Figures 10A-C are photographs showing monolayer cultures of bovine endothelial cells in the presence (Figure 10A) and absence (Figure 10C) of a

representative cyclic peptide or in the presence of an inactive control peptide (Figure 10B). Figure 10A shows the cells 30 minutes after exposure to 500 μg/mL N-Ac-CSHAVSSC-NH$_2$ (SEQ ID NO:18). Figure 10B shows the cells 30 minutes after exposure to the control peptide N-Ac-CSHGVSSC-NH$_2$ (SEQ ID NO:19). Figure 10C shows the cells in the absence of cyclic peptide. Note that the endothelial cells retracted from one another and round up in the presence of N-Ac-CSHAVSSC-NH$_2$ (SEQ ID NO:18).

Figures 11A-F are photographs showing monolayer cultures of human ovarian cancer cells (SKOV3) in the presence (Figures 11A and D-F) and absence (Figure 11C) of a representative cyclic peptide or in the presence of an inactive control peptide (Figure 11B). Figure 11A shows the cells 24 hours after being cultured in the presence of 500 μg/mL N-Ac-CHAVC-NH$_2$ (10X magnification). Figure 11B shows the cells (10X magnification) 24 hours after being cultured in the presence of the control peptide N-Ac-CHGVC-NH$_2$ (SEQ ID NO:9). Figure 11C shows the cells (10X magnification) in the absence of cyclic peptide. Figures 11D-F show the cells (20X magnification) 48 hours after exposure to N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8) at concentrations of 1 mg/mL, 100 μg/mL and 10 μg/mL, respectively. Note that the SKOV3 cells retract from one another and round-up when cultured in the presence of either 0.5 or 1mg/ml N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8).

Figures 12A and 12B are photographs showing monolayer cultures of human ovarian cancer cells (SKOV3) 24 hours after exposure to 500μg/mL of the representative cyclic peptide N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8) (Figure 12A) or the control peptide N-Ac-CHGVC-NH$_2$ (Figure 12B). Note that the SKOV3 cells round-up when cultured in the presence of 0.5 mg/ml N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8).

Figures 13A-D are photographs of monolayer cultures of normal rat kidney (NRK) cells untreated (Figure 13A) or after 48 hours of exposure to 1 mg/mL H-CHAVSC-OH (SEQ ID NO:14) (Figure 13B), the control peptide N-Ac-CHGVC-NH$_2$ (SEQ ID NO:9), (Figure 13C) or the representative cyclic peptide N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8), (Figure 13D). Note that NRK cells retract from one another when

cultured in the presence of N-Ac-CHAVC-NH_2 (SEQ ID NO:8). Furthermore the NRK cells do not form cobblestone-like monolayers when exposed to this peptide.

Figures 14A-D are immunofluorescence photographs of the monolayer normal rat kidney (NRK) cultures shown in Figures 13A-D immunolabeled for E-cadherin. Figure 14A shows untreated cells and Figures 14B-D show cells after 48 hours of exposure to either 1 mg/mL H-CHAVSC-OH (SEQ ID NO:14) (Figure 14B), the control peptide N-Ac-CHGVC-NH_2 (SEQ ID NO:9), (Figure 14C) or the representative cyclic peptide N-Ac-CHAVC-NH_2 (SEQ ID NO:8), (Figure 14D). Note that E-cadherin expression is greatly reduced in the cells treated with N-Ac-CHAVC-NH_2 (SEQ ID NO:8), as compared to the E-cadherin levels expressed by untreated cells and cells treated with the other two cyclic peptides

Figures 15A-C are photographs showing monolayer cultures of human ovarian cancer cells (OVCAR3) in the presence of varying concentrations of a representative cyclic peptide. Figure 15A shows the cells 24 hours after being cultured in the presence of 1 mg/ml of N-Ac-CHAVSC-NH_2 (SEQ ID NO:14). Figure 15B shows the cells 24 hours after being cultured in the presence of 100 μg/ml of N-Ac-CHAVSC-NH_2 (SEQ ID NO:14). Figure 15C shows the cells 24 hours after being cultured in the presence of 10 μg/ml of N-Ac-CHAVSC-NH_2 (SEQ ID NO:14). Note that the cells retract form one another in the presence of 100 μg/ml of N-Ac-CHAVSC-NH_2 (SEQ ID NO:14), whereas they round up in the presence of 1 mg/ml of this peptide.

Figures 16A and B are photographs showing cultures of human melanoma ME115 cells in the presence (Figure 16B) and absence (Figure 16A) of a representative cyclic peptide. The cells have been immunolabeled for cadherin. Figure 16B shows the cells 48 hours after being cultured in the presence of 500 μg/ml of N-Ac-CHAVC-NH_2 (SEQ ID NO:8). Figure 16A shows untreated cultures of human melanoma ME115 cells. Note that cadherin is localized in intracellular vesicles in cells treated with peptide, whereas it is present at the surface in the untreated cells.

Figures 17A and B are photographs showing monolayer cultures of A1N4 human breast epithelial cells in the presence (Figure 17B) and absence (Figure

17A) of a representative cyclic peptide. The cells have been immunolabeled for E-cadherin. Figure 17B shows the cells 48 hours after being cultured in the presence of 500 µg/ml of N-Ac-CHAVC-NH_2 (SEQ ID NO:8). Figure 17A shows untreated monolayer cultures of A1N4 human breast epithelial cells. Note that the distribution of E-cadherin is non-contiguous in cells treated with the cyclic peptide. Furthermore, gaps have appeared in the monolayer of cells treated with the peptide.

Figure 18 is a histogram illustrating the effect of 500 µg/ml of a representative cyclic peptide (N-Ac-CHAVC-NH_2; treatment bars) on the penetration of Oregon Green through the skin, as compared to the effect of the control peptide N-Ac-CHGVC-NH_2 (control bars). Penetration was determined by converting fluorescent units to a concentration unit of microgram/5ml (volume of the receiver compartment) using a standard curve and regression analysis equations.

Figure 19 is a histogram illustrating the effect of 500 µg/ml of a representative cyclic peptide (N-Ac-CHAVC-NH_2; treatment bars) on the penetration of Rhodamine Green through the skin, as compared to the effect of the control peptide N-Ac-CHGVC-NH_2 (control bars). Penetration was determined by converting fluorescent units to a concentration unit of microgram/5ml (volume of the receiver compartment) using a standard curve and regression analysis equations.

Figure 20 is a histogram illustrating the effect of 2.5 mg/ml of a representative cyclic peptide (N-Ac-CHAVC-NH_2; treatment bars) on the penetration of Oregon Green through the skin, as compared to the effect of the control peptide N-Ac-CHGVC-NH_2 (control bars). Penetration was determined by converting fluorescent units to a concentration unit of microgram/5ml (volume of the receiver compartment) using a standard curve and regression analysis equations.

Figure 21 is a histogram illustrating the effect of 2.5 mg/ml of a representative cyclic peptide (N-Ac-CHAVC-NH_2; treatment bars) on the penetration of Rhodamine Green through the skin, as compared to the effect of the control peptide N-Ac-CHGVC-NH_2 (control bars). Penetration was determined by converting fluorescent units to a concentration unit of microgram/5ml (volume of the receiver compartment) using a standard curve and regression analysis equations.

Figure 22 is a graph illustrating the results of a study to assess the chronic toxicity of a representative cyclic peptide. The graph presents the mean body weight during the three-day treatment period (one intraperitoneal injection per day) and the four subsequent recovery days. Three different doses are illustrated, as indicated.

Figure 23 is a graph illustrating the stability of a representative cyclic peptide in mouse whole blood. The percent of the cyclic peptide remaining in the blood was assayed at various time points, as indicated.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention provides cell adhesion modulating agents comprising cyclic peptides that are capable of modulating cadherin-mediated processes, such as cell adhesion. In general, to modulate cadherin-mediated cell adhesion, a cadherin-expressing cell is contacted with a cell adhesion modulating agent (also referred to herein as a "modulating agent") either *in vivo* or *in vitro*. A modulating agent comprises a cyclic peptide that contains the classical cadherin cell adhesion recognition (CAR) sequence HAV (*i.e.*, His-Ala-Val). Such modulating agents may further comprise one or more additional CAR sequences and/or an antibody (or antigen-binding fragment thereof) that specifically binds to a cadherin CAR sequence, as described below. Certain cell adhesion modulating agents (also referred to herein as "modulating agents") described herein inhibit cell adhesion. Such modulating agents may generally be used, for example, to treat diseases or other conditions characterized by undesirable cell adhesion or to facilitate drug delivery to a specific tissue or tumor. Alternatively, certain modulating agents may be used to enhance cell adhesion (*e.g.*, to supplement or replace stitches or to facilitate wound healing) or to enhance or direct neurite outgrowth.

CELL ADHESION MODULATING AGENTS

The term "cell adhesion modulating agent," as used herein, refers to a molecule comprising at least one cyclic peptide that contains a cadherin cell adhesion recognition (CAR) sequence, generally HAV (His-Ala-Val). The term "cyclic peptide,"

as used herein, refers to a peptide or salt thereof that comprises (1) an intramolecular covalent bond between two non-adjacent residues and (2) at least one cadherin CAR sequence. The intramolecular bond may be a backbone to backbone, side-chain to backbone or side-chain to side-chain bond (*i.e.*, terminal functional groups of a linear peptide and/or side chain functional groups of a terminal or interior residue may be linked to achieve cyclization). Preferred intramolecular bonds include, but are not limited to, disulfide, amide and thioether bonds. In addition to the cadherin CAR sequence HAV, a modulating agent may comprise additional CAR sequences, which may or may not be cadherin CAR sequences, and/or antibodies or fragments thereof that specifically recognize a CAR sequence. Additional CAR sequences may be present within the cyclic peptide containing the HAV sequence, within a separate cyclic peptide component of the modulating agent and/or in a non-cyclic portion of the modulating agent. Antibodies and antigen-binding fragments thereof are typically present in a non-cyclic portion of the modulating agent.

In addition to the CAR sequence(s), cyclic peptides generally comprise at least one additional residue, such that the size of the cyclic peptide ring ranges from 4 to about 15 residues, preferably from 5 to 10 residues. Such additional residue(s) may be present on the N-terminal and/or C-terminal side of a CAR sequence, and may be derived from sequences that flank the HAV sequence within one or more naturally occurring cadherins (*e.g.*, N-cadherin, E-cadherin, P-cadherin, R-cadherin or other cadherins containing the HAV sequence) with or without amino acid substitutions and/or other modifications. Flanking sequences for endogenous N-, E-, P- and R-cadherin are shown in Figure 2, and in SEQ ID NOs: 1 to 7. Database accession numbers for representative naturally occurring cadherins are as follows: human N-cadherin M34064, mouse N-cadherin M31131 and M22556, cow N-cadherin X53615, human P-cadherin X63629, mouse P-cadherin X06340, human E-cadherin Z13009, mouse E-cadherin X06115. Alternatively, additional residues present on one or both sides of the CAR sequence(s) may be unrelated to an endogenous sequence (*e.g.*, residues that facilitate cyclization).

Within certain preferred embodiments, as discussed below, relatively small cyclic peptides that do not contain significant sequences flanking the HAV sequence are preferred for modulating N-cadherin and E-cadherin mediated cell adhesion. Such peptides may contain an N-acetyl group and a C-amide group (*e.g.*, the 5-residue ring N-Ac-<u>CHAVC</u>-NH_2 (SEQ ID NO:8) or N-Ac-<u>KHAVD</u>-NH_2 (SEQ ID NO:20)). The finding, within the present invention, that such relatively small cyclic peptides may be effective and all-purpose inhibitors of cell adhesion represents a unexpected discovery. Such cyclic peptides can be thought of as "master keys" that fit into peptide binding sites of each of the different classical cadherins, and are capable of disrupting cell adhesion of neural cells, endothelial cells, epithelial cells and/or certain cancer cells. Small cyclic peptides may generally be used to specifically modulate cell adhesion of neural and/or other cell types by topical administration or by systemic administration, with or without linking a targeting agent to the peptide, as discussed below.

Within other preferred embodiments, a cyclic peptide may contain sequences that flank the HAV sequence on one or both sides that are designed to confer specificity for cell adhesion mediated by one or more specific cadherins, resulting in tissue and/or cell-type specificity. Suitable flanking sequences for conferring specificity include, but are not limited to, endogenous sequences present in one or more naturally occurring cadherins, and cyclic peptides having specificity may be identified using the representative screens provided herein. For example, it has been found, within the context of the present invention, that cyclic peptides that contain additional residues derived from the native E-cadherin sequence on the C-terminal side of the CAR sequence are specific for epithelial cells (*i.e.*, such peptides disrupt E-cadherin mediated cell adhesion to a greater extent than they disrupt N-cadherin expression). The addition of appropriate endogenous sequences may similarly result in peptides that disrupt N-cadherin mediated cell adhesion.

To facilitate the preparation of cyclic peptides having a desired specificity, nuclear magnetic resonance (NMR) and computational techniques may be used to determine the conformation of a peptide that confers a known specificity. NMR

is widely used for structural analysis of molecules. Cross-peak intensities in nuclear Overhauser enhancement (NOE) spectra, coupling constants and chemical shifts depend on the conformation of a compound. NOE data provide the interproton distance between protons through space and across the ring of the cyclic peptide. This information may be used to facilitate calculation of the lowest energy conformation for the HAV sequence. Conformation may then be correlated with tissue specificity to permit the identification of peptides that are similarly tissue specific or have enhanced tissue specificity.

As noted above, multiple CAR sequences may be present within a modulating agent. CAR sequences that may be included within a modulating agent are any sequences specifically bound by an adhesion molecule. As used herein, an "adhesion molecule" is any molecule that mediates cell adhesion via a receptor on the cell's surface. Adhesion molecules include members of the cadherin gene superfamily that are not classical cadherins (*e.g.*, proteins that do not contain an HAV sequence and/or one or more of the other characteristics recited above for classical cadherins), such as desmogleins (Dsg) and desmocollins (Dsc); integrins; members of the immunoglobulin supergene family, such as N-CAM; and other uncategorized transmembrane proteins, such as occludin, as well as extracellular matrix proteins such as laminin, fibronectin, collagens, vitronectin, entactin and tenascin. Preferred CAR sequences for inclusion within a modulating agent include Arg-Gly-Asp (RGD), which is bound by integrins (*see* Cardarelli et al., *J. Biol. Chem.* *267*:23159-64, 1992); Tyr-Ile-Gly-Ser-Arg (YIGSR; SEQ ID NO:47), which is bound by $\alpha6\beta1$ integrin; KYSFNYDGSE (SEQ ID NO:62), which is bound by N-CAM; the N-CAM heparin sulfate-binding site IWKHKGRDVILKKDVRF (SEQ ID NO:63); the putative Dsc CAR sequences YAT, FAT and YAS; the putative Dsg CAR sequence RAL; and/or the putative occludin CAR sequence GVNPTAQSSGSLYGSQIYALCNQFYTPAAT-GLYVDQYLYHYCVVDPQE (SEQ ID NO:59), or derivatives or portions thereof such as QYLYHYCVVD (SEQ ID NO:60) and LYHY (SEQ ID NO:58).

Linkers may, but need not, be used to separate CAR sequences and/or antibody sequences within a modulating agent. Linkers may also, or alternatively, be

used to attach one or more modulating agents to a support molecule or material, as described below. A linker may be any molecule (including peptide and/or non-peptide sequences as well as single amino acids or other molecules), that does not contain a CAR sequence and that can be covalently linked to at least two peptide sequences. Using a linker, HAV-containing cyclic peptides and other peptide or protein sequences may be joined head-to-tail (*i.e.*, the linker may be covalently attached to the carboxyl or amino group of each peptide sequence), head-to-side chain and/or tail-to-side chain. Modulating agents comprising one or more linkers may form linear or branched structures. Within one embodiment, modulating agents having a branched structure comprise three different CAR sequences, such as RGD, YIGSR (SEQ ID NO:47) and HAV, one or more of which are present within a cyclic peptide. Within another embodiment, modulating agents having a branched structure comprise RGD, YIGSR (SEQ ID NO:47), HAV and KYSFNYDGSE (SEQ ID NO:62). In a third embodiment, modulating agents having a branched structure comprise HAV, YAT, FAT, YAS, LYHY (SEQ ID NO:58) and RAL. Bi-functional modulating agents that comprise an HAV sequence with flanking E-cadherin-specific sequences joined via a linker to an HAV sequence with flanking N-cadherin-specific sequences are also preferred for certain embodiments.

Linkers preferably produce a distance between CAR sequences between 0.1 to 10,000 nm, more preferably about 0.1-400 nm. A separation distance between recognition sites may generally be determined according to the desired function of the modulating agent. For inhibitors of cell adhesion, the linker distance should be small (0.1-400 nm). For enhancers of cell adhesion, the linker distance should be 400-10,000 nm. One linker that can be used for such purposes is $(H_2N(CH_2)_nCO_2H)_m$, or derivatives thereof, where n ranges from 1 to 10 and m ranges from 1 to 4000. For example, if glycine ($H_2NCH_2CO_2H$) or a multimer thereof is used as a linker, each glycine unit corresponds to a linking distance of 2.45 angstroms, or 0.245 nm, as determined by calculation of its lowest energy conformation when linked to other amino acids using molecular modeling techniques. Similarly, aminopropanoic acid corresponds to a linking distance of 3.73 angstroms, aminobutanoic acid to 4.96 angstroms,

aminopentanoic acid to 6.30 angstroms and amino hexanoic acid to 6.12 angstroms. Other linkers that may be used will be apparent to those of ordinary skill in the art and include, for example, linkers based on repeat units of 2,3-diaminopropanoic acid, lysine and/or ornithine. 2,3-Diaminopropanoic acid can provide a linking distance of either 2.51 or 3.11 angstroms depending on whether the side-chain amino or terminal amino is used in the linkage. Similarly, lysine can provide linking distances of either 2.44 or 6.95 angstroms and ornithine 2.44 or 5.61 angstroms. Peptide and non-peptide linkers may generally be incorporated into a modulating agent using any appropriate method known in the art.

Modulating agents that inhibit cell adhesion typically contain one HAV sequence or multiple HAV sequences, which may be adjacent to one another (*i.e.*, without intervening sequences) or in close proximity (*i.e.*, separated by peptide and/or non-peptide linkers to give a distance between the CAR sequences that ranges from about 0.1 to 400 nm). Within one such embodiment, the cyclic peptide contains two HAV sequences. Such a modulating agent may additionally comprise a CAR sequence for one or more different adhesion molecules (including, but not limited to, other CAMs) and/or one or more antibodies or fragments thereof that bind to such sequences. Linkers may, but need not, be used to separate such CAR sequence(s) and/or antibody sequence(s) from the HAV sequence(s) and/or each other. Such modulating agents may generally be used within methods in which it is desirable to simultaneously disrupt cell adhesion mediated by multiple adhesion molecules. Within certain preferred embodiments, the second CAR sequence is derived from fibronectin and is recognized by an integrin (*i.e.*, RGD; *see* Cardarelli et al., *J. Biol. Chem. 267*:23159-23164, 1992), or is an occludin CAR sequence (*e.g.*, LYHY; SEQ ID NO:58). One or more antibodies, or fragments thereof, may similarly be used within such embodiments.

Modulating agents that enhance cell adhesion may contain multiple HAV sequences, and/or antibodies that specifically bind to such sequences, joined by linkers as described above. Enhancement of cell adhesion may also be achieved by attachment of multiple modulating agents to a support molecule or material, as discussed further below. Such modulating agents may additionally comprise one or

more CAR sequence for one or more different adhesion molecules (including, but not limited to, other CAMs) and/or one or more antibodies or fragments thereof that bind to such sequences, to enhance cell adhesion mediated by multiple adhesion molecules.

Modulating agents and cyclic peptides as described herein may comprise residues of L-amino acids, D-amino acids, or any combination thereof. Amino acids may be from natural or non-natural sources, provided that at least one amino group and at least one carboxyl group are present in the molecule; α- and β-amino acids are generally preferred. The 20 L-amino acids commonly found in proteins are identified herein by the conventional three-letter or one-letter abbreviations indicated in Table 1, and the corresponding D-amino acids are designated by a lower case one letter symbol. Modulating agents and cyclic peptides may also contain one or more rare amino acids (such as 4-hydroxyproline or hydroxylysine), organic acids or amides and/or derivatives of common amino acids, such as amino acids having the C-terminal carboxylate esterified (*e.g.*, benzyl, methyl or ethyl ester) or amidated and/or having modifications of the N-terminal amino group (*e.g.*, acetylation or alkoxycarbonylation), with or without any of a wide variety of side-chain modifications and/or substitutions (*e.g.*, methylation, benzylation, t-butylation, tosylation, alkoxycarbonylation, and the like). Preferred derivatives include amino acids having an N-acetyl group (such that the amino group that represents the N-terminus of the linear peptide prior to cyclization is acetylated) and/or a C-terminal amide group (*i.e.*, the carboxy terminus of the linear peptide prior to cyclization is amidated). Residues other than common amino acids that may be present with a cyclic peptide include, but are not limited to, penicillamine, β,β-tetramethylene cysteine, β,β-pentamethylene cysteine, β-mercaptopropionic acid, β,β-pentamethylene-β-mercaptopropionic acid, 2-mercaptobenzene, 2-mercaptoaniline, 2-mercaptoproline, ornithine, diaminobutyric acid, α-aminoadipic acid, m-aminomethylbenzoic acid and α,β-diaminopropionic acid.

Table 1

Amino acid one-letter and three-letter abbreviations

A	Ala	Alanine
R	Arg	Arginine
D	Asp	Aspartic acid
N	Asn	Asparagine
C	Cys	Cysteine
Q	Gln	Glutamine
E	Glu	Glutamic acid
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
L	Leu	Leucine
K	Lys	Lysine
M	Met	Methionine
F	Phe	Phenylalanine
P	Pro	Proline
S	Ser	Serine
T	Thr	Threonine
W	Trp	Tryptophan
Y	Tyr	Tyrosine
V	Val	Valine

Modulating agents and cyclic peptides as described herein may be synthesized by methods well known in the art, including recombinant DNA methods and chemical synthesis. Chemical synthesis may generally be performed using standard solution phase or solid phase peptide synthesis techniques, in which a peptide linkage occurs through the direct condensation of the α-amino group of one amino acid with the α-carboxy group of the other amino acid with the elimination of a water molecule. Peptide bond synthesis by direct condensation, as formulated above, requires suppression of the reactive character of the amino group of the first and of the carboxyl group of the second amino acid. The masking substituents must permit their ready removal, without inducing breakdown of the labile peptide molecule.

In solution phase synthesis, a wide variety of coupling methods and protecting groups may be used (*see* Gross and Meienhofer, eds., "The Peptides: Analysis, Synthesis, Biology," Vol. 1-4 (Academic Press, 1979); Bodansky and Bodansky, "The Practice of Peptide Synthesis," 2d ed. (Springer Verlag, 1994)). In addition, intermediate purification and linear scale up are possible. Those of ordinary skill in the art will appreciate that solution synthesis requires consideration of main chain and side chain protecting groups and activation method. In addition, careful segment selection is necessary to minimize racemization during segment condensation. Solubility considerations are also a factor.

Solid phase peptide synthesis uses an insoluble polymer for support during organic synthesis. The polymer-supported peptide chain permits the use of simple washing and filtration steps instead of laborious purifications at intermediate steps. Solid-phase peptide synthesis may generally be performed according to the method of Merrifield et al., *J. Am. Chem. Soc. 85*:2149, 1963, which involves assembling a linear peptide chain on a resin support using protected amino acids. Solid phase peptide synthesis typically utilizes either the Boc or Fmoc strategy. The Boc strategy uses a 1% cross-linked polystyrene resin. The standard protecting group for α-amino functions is the tert-butyloxycarbonyl (Boc) group. This group can be removed with dilute solutions of strong acids such as 25% trifluoroacetic acid (TFA). The next Boc-amino acid is typically coupled to the amino acyl resin using dicyclohexylcarbodiimide (DCC). Following completion of the assembly, the peptide-resin is treated with anhydrous HF to cleave the benzyl ester link and liberate the free peptide. Side-chain functional groups are usually blocked during synthesis by benzyl-derived blocking groups, which are also cleaved by HF. The free peptide is then extracted from the resin with a suitable solvent, purified and characterized. Newly synthesized peptides can be purified, for example, by gel filtration, HPLC, partition chromatography and/or ion-exchange chromatography, and may be characterized by, for example, mass spectrometry or amino acid sequence analysis. In the Boc strategy, C-terminal amidated peptides can be obtained using benzhydrylamine or

methylbenzhydrylamine resins, which yield peptide amides directly upon cleavage with HF.

In the procedures discussed above, the selectivity of the side-chain blocking groups and of the peptide-resin link depends upon the differences in the rate of acidolytic cleavage. Orthoganol systems have been introduced in which the side-chain blocking groups and the peptide-resin link are completely stable to the reagent used to remove the α-protecting group at each step of the synthesis. The most common of these methods involves the 9-fluorenylmethyloxycarbonyl (Fmoc) approach. Within this method, the side-chain protecting groups and the peptide-resin link are completely stable to the secondary amines used for cleaving the N-α-Fmoc group. The side-chain protection and the peptide-resin link are cleaved by mild acidolysis. The repeated contact with base makes the Merrifield resin unsuitable for Fmoc chemistry, and p-alkoxybenzyl esters linked to the resin are generally used. Deprotection and cleavage are generally accomplished using TFA.

Those of ordinary skill in the art will recognize that, in solid phase synthesis, deprotection and coupling reactions must go to completion and the side-chain blocking groups must be stable throughout the entire synthesis. In addition, solid phase synthesis is generally most suitable when peptides are to be made on a small scale.

Acetylation of the N-terminal can be accomplished by reacting the final peptide with acetic anhydride before cleavage from the resin. C-amidation is accomplished using an appropriate resin such as methylbenzhydrylamine resin using the Boc technology.

Following synthesis of a linear peptide, with or without N-acetylation and/or C-amidation, cyclization may be achieved by any of a variety of techniques well known in the art. Within one embodiment, a bond may be generated between reactive amino acid side chains. For example, a disulfide bridge may be formed from a linear peptide comprising two thiol-containing residues by oxidizing the peptide using any of a variety of methods. Within one such method, air oxidation of thiols can generate disulfide linkages over a period of several days using either basic or neutral aqueous media. The peptide is used in high dilution to minimize aggregation and intermolecular

side reactions. This method suffers from the disadvantage of being slow but has the advantage of only producing H_2O as a side product. Alternatively, strong oxidizing agents such as I_2 and $K_3Fe(CN)_6$ can be used to form disulfide linkages. Those of ordinary skill in the art will recognize that care must be taken not to oxidize the sensitive side chains of Met, Tyr, Trp or His. Cyclic peptides produced by this method require purification using standard techniques, but this oxidation is applicable at acid pHs. By way of example, strong oxidizing agents can be used to perform the cyclization shown below (SEQ ID NOs: 33 and 34), in which the underlined portion is cyclized:

FmocCysAsp(t-Bu)GlyTyr(t-Bu)ProLys(Boc)Asp(t-Bu)CysLys(t-Bu)Gly-OMe
→
Fmoc<u>CysAsp(t-Bu)GlyTyr(t-Bu)ProLys(Boc)Asp(t-Bu)Cys</u>Lys(t-Bu)Gly-OMe

Oxidizing agents also allow concurrent deprotection/oxidation of suitable S-protected linear precursors to avoid premature, nonspecific oxidation of free cysteine, as shown below (SEQ ID NOs: 35 and 36), where X and Y = S-Trt or S-Acm:

BocCys(X)GlyAsnLeuSer(t-Bu)Thr(t-Bu)Cys(Y)MetLeuGlyOH →
Boc<u>CysGlyAsnLeuSer(t-Bu)Thr(t-Bu)Cys</u>MetLeuGlyOH

DMSO, unlike I_2 and $K_3Fe(CN)_6$, is a mild oxidizing agent which does not cause oxidative side reactions of the nucleophilic amino acids mentioned above. DMSO is miscible with H_2O at all concentrations, and oxidations can be performed at acidic to neutral pHs with harmless byproducts. Methyltrichlorosilane-diphenylsulfoxide may alternatively be used as an oxidizing agent, for concurrent deprotection/oxidation of S-Acm, S-Tacm or S-t-Bu of cysteine without affecting other nucleophilic amino acids. There are no polymeric products resulting from intermolecular disulfide bond formation. In the example below (SEQ ID NOs: 37 and 38), X is Acm, Tacm or t-Bu:

H-Cys(X)TyrIleGlnAsnCys(X)ProLeuGly-NH$_2$ $\rightarrow$
H-<u>CysTyrIleGlnAsnCys</u>ProLeuGly-NH$_2$

Suitable thiol-containing residues for use in such oxidation methods include, but are not limited to, cysteine, β,β-dimethyl cysteine (penicillamine or Pen), β,β-tetramethylene cysteine (Tmc), β,β-pentamethylene cysteine (Pmc), β-mercaptopropionic acid (Mpr), β,β-pentamethylene-β-mercaptopropionic acid (Pmp), 2-mercaptobenzene, 2-mercaptoaniline and 2-mercaptoproline. Peptides containing such residues are illustrated by the following representative formulas, in which the underlined portion is cyclized, N-acetyl groups are indicated by N-Ac and C-terminal amide groups are represented by -NH$_2$:

i) N-Ac-<u>Cys-His-Ala-Val-Cys</u>-NH$_2$ (SEQ ID NO:8)

ii) N-Ac-<u>Cys-Ala-His-Ala-Val-Asp-Ile-Cys</u>-NH$_2$ (SEQ ID NO:10)

iii) N-Ac-<u>Cys-Ser-His-Ala-Val-Cys</u>-NH$_2$ (SEQ ID NO:12)

iv) N-Ac-<u>Cys-His-Ala-Val-Ser-Cys</u>-NH$_2$ (SEQ ID NO:14)

v) N-Ac-<u>Cys-Ala-His-Ala-Val-Asp-Cys</u>-NH$_2$ (SEQ ID NO:16)

vi) N-Ac-<u>Cys-Ser-His-Ala-Val-Ser-Ser-Cys</u>-NH$_2$ (SEQ ID NO:18)

vii) N-Ac-<u>Cys-His-Ala-Val-Ser-Cys</u>-OH (SEQ ID NO:14)

viii) H-<u>Cys-Ala-His-Ala-Val-Asp-Cys</u>-NH$_2$ (SEQ ID NO:16)

ix) N-Ac-<u>Cys-His-Ala-Val-Pen</u>-NH$_2$ (SEQ ID NO:28)

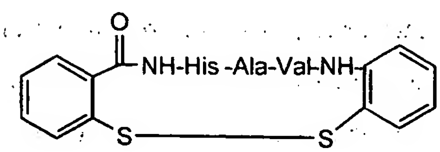
x) N-Ac-Ile-<u>Tmc-Tyr-Ser-His-Ala-Val-Ser-Cys</u>-Glu-NH_2 (SEQ ID NO:29)

xi) N-Ac-Ile-<u>Pmc-Tyr-Ser-His-Ala-Val-Ser-Ser-Cys</u>-NH_2 (SEQ ID NO:30)

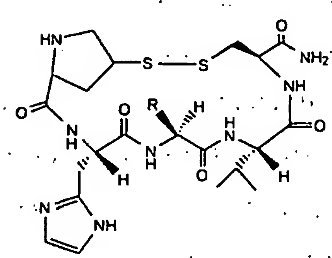
xii) <u>Mpr-Tyr-Ser-His-Ala-Val-Ser-Ser-Cys</u>-NH_2 (SEQ ID NO:31)

xiii) <u>Pmp-Tyr-Ser-His-Ala-Val-Ser-Ser-Cys</u>-NH_2 (SEQ ID NO:32)

xii)



xiii)



It will be readily apparent to those of ordinary skill in the art that, within each of these representative formulas, any of the above thiol-containing residues may be employed in place of one or both of the thiol-containing residues recited.

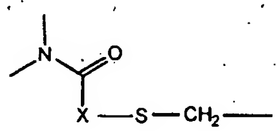
Within another embodiment, cyclization may be achieved by amide bond formation. For example, a peptide bond may be formed between terminal functional groups (*i.e.*, the amino and carboxy termini of a linear peptide prior to cyclization). Two such cyclic peptides are <u>AHAVDI</u> (SEQ ID NO:44) and <u>SHAVSS</u> (SEQ ID NO:45), with or without an N-terminal acetyl group and/or a C-terminal amide. Within another such embodiment, the linear peptide comprises a D-amino acid (*e.g.*, <u>HAVsS</u>).

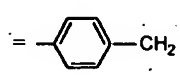
Alternatively, cyclization may be accomplished by linking one terminus and a residue side chain or using two side chains, as in KHAVD (SEQ ID NO:20) or KSHAVSSD (SEQ ID NO:46), with or without an N-terminal acetyl group and/or a C-terminal amide. Residues capable of forming a lactam bond include lysine, ornithine (Orn), α-amino adipic acid, m-aminomethylbenzoic acid, α,β-diaminopropionic acid, glutamate or aspartate.

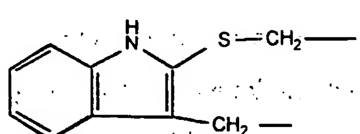
Methods for forming amide bonds are well known in the art and are based on well established principles of chemical reactivity. Within one such method, carbodiimide-mediated lactam formation can be accomplished by reaction of the carboxylic acid with DCC, DIC, EDAC or DCCI, resulting in the formation of an O-acylurea that can be reacted immediately with the free amino group to complete the cyclization. The formation of the inactive N-acylurea, resulting from O→N migration, can be circumvented by converting the O-acylurea to an active ester by reaction with an N-hydroxy compound such as 1-hydroxybenzotriazole, 1-hydroxysuccinimide, 1-hydroxynorbornene carboxamide or ethyl 2-hydroximino-2-cyanoacetate. In addition to minimizing O→N migration, these additives also serve as catalysts during cyclization and assist in lowering racemization. Alternatively, cyclization can be performed using the azide method, in which a reactive azide intermediate is generated from an alkyl ester via a hydrazide. Hydrazinolysis of the terminal ester necessitates the use of a t-butyl group for the protection of side chain carboxyl functions in the acylating component. This limitation can be overcome by using diphenylphosphoryl acid (DPPA), which furnishes an azide directly upon reaction with a carboxyl group. The slow reactivity of azides and the formation of isocyanates by their disproportionation restrict the usefulness of this method. The mixed anhydride method of lactam formation is widely used because of the facile removal of reaction by-products. The anhydride is formed upon reaction of the carboxylate anion with an alkyl chloroformate or pivaloyl chloride. The attack of the amino component is then guided to the carbonyl carbon of the acylating component by the electron donating effect of the alkoxy group or by the steric bulk of the pivaloyl chloride t-butyl group, which obstructs attack on the wrong carbonyl group. Mixed anhydrides with phosphoric acid derivatives have also been

successfully used. Alternatively, cyclization can be accomplished using activated esters. The presence of electron withdrawing substituents on the alkoxy carbon of esters increases their susceptibility to aminolysis. The high reactivity of esters of p-nitrophenol, N-hydroxy compounds and polyhalogenated phenols has made these "active esters" useful in the synthesis of amide bonds. The last few years have witnessed the development of benzotriazolyloxytris-(dimethylamino)phosphonium hexafluorophosphonate (BOP) and its congeners as advantageous coupling reagents. Their performance is generally superior to that of the well established carbodiimide amide bond formation reactions.

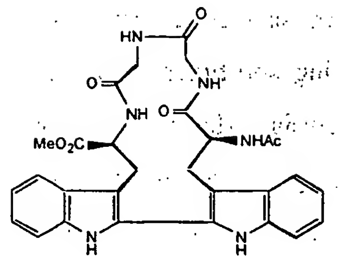
Within a further embodiment, a thioether linkage may be formed between the side chain of a thiol-containing residue and an appropriately derivatized α-amino acid. By way of example, a lysine side chain can be coupled to bromoacetic acid through the carbodiimide coupling method (DCC, EDAC) and then reacted with the side chain of any of the thiol containing residues mentioned above to form a thioether linkage. In order to form dithioethers, any two thiol containing side-chains can be reacted with dibromoethane and diisopropylamine in DMF. Examples of thiol-containing linkages are shown below:

i. 

$X = (CH_2)_4$
$= CH_2$


ii. 

Cyclization may also be achieved using δ_1,δ_1-Ditryptophan (*i.e.*, Ac-Trp-Gly-Gly-Trp-OMe) (SEQ ID NO:39), as shown below:



Representative structures of cyclic peptides are provided in Figure 3. Within Figure 3, certain cyclic peptides having the ability to modulate cell adhesion (shown on the left) are paired with similar inactive structures (on the right). The structures and formulas recited herein are provided solely for the purpose of illustration, and are not intended to limit the scope of the cyclic peptides described herein.

As noted above, a modulating agent may consist entirely of one or more cyclic peptides, or may contain additional peptide and/or non-peptide sequences, which may be linked to the cyclic peptide(s) using conventional techniques. Peptide portions may be synthesized as described above or may be prepared using recombinant methods. Within such methods, all or part of a modulating agent can be synthesized in living cells, using any of a variety of expression vectors known to those of ordinary skill in the art to be appropriate for the particular host cell. Suitable host cells may include bacteria, yeast cells, mammalian cells, insect cells, plant cells, algae and other animal cells (*e.g.*, hybridoma, CHO, myeloma). The DNA sequences expressed in this manner may encode portions of an endogenous cadherin or other adhesion molecule. Such sequences may be prepared based on known cDNA or genomic sequences (*see* Blaschuk et al., *J. Mol. Biol. 211*:679-682, 1990), or from sequences isolated by screening an appropriate library with probes designed based on the sequences of known cadherins. Such screens may generally be performed as described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989 (and references cited therein). Polymerase chain reaction (PCR) may also be employed, using oligonucleotide primers in methods well known in

the art, to isolate nucleic acid molecules encoding all or a portion of an endogenous adhesion molecule. To generate a nucleic acid molecule encoding a peptide portion of a modulating agent, an endogenous sequence may be modified using well known techniques. For example, portions encoding one or more CAR sequences may be joined, with or without separation by nucleic acid regions encoding linkers, as discussed above. Alternatively, portions of the desired nucleic acid sequences may be synthesized using well known techniques, and then ligated together to form a sequence encoding a portion of the modulating agent.

As noted above, portions of a modulating agent may comprise an antibody, or antigen-binding fragment thereof, that specifically binds to a CAR sequence. As used herein, an antibody, or antigen-binding fragment thereof, is said to "specifically bind" to a CAR sequence (with or without flanking amino acids) if it reacts at a detectable level (within, for example, an ELISA, as described by Newton et al., *Develop. Dynamics 197*:1-13, 1993) with a peptide containing that sequence, and does not react detectably with peptides containing a different CAR sequence or a sequence in which the order of amino acid residues in the cadherin CAR sequence and/or flanking sequence is altered.

Antibodies and fragments thereof may be prepared using standard techniques. *See, e.g.,* Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In one such technique, an immunogen comprising a CAR sequence is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep or goats). Small immunogens (*i.e.*, less than about 20 amino acids) should be joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. Following one or more injections, the animals are bled periodically. Polyclonal antibodies specific for the CAR sequence may then be purified from such antisera by, for example, affinity chromatography using the modulating agent or antigenic portion thereof coupled to a suitable solid support.

Monoclonal antibodies specific for a CAR sequence may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol. 6*:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of

immortal cell lines capable of producing antibodies having the desired specificity from spleen cells obtained from an animal immunized as described above. The spleen cells are immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. Single colonies are selected and their culture supernatants tested for binding activity against the modulating agent or antigenic portion thereof. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies, with or without the use of various techniques known in the art to enhance the yield. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. Antibodies having the desired activity may generally be identified using immunofluorescence analyses of tissue sections, cell or other samples where the target cadherin is localized.

Within certain embodiments, monoclonal antibodies may be specific for particular cadherins (*e.g.*, the antibodies bind to E-cadherin, but do not bind significantly to N-cadherin, or vise versa). Such antibodies may be prepared as described above, using an immunogen that comprises (in addition to the HAV sequence) sufficient flanking sequence to generate the desired specificity (*e.g.*, 5 amino acids on each side is generally sufficient). One representative immunogen is the 15-mer FHLRAHAVDINGNQV-NH_2 (SEQ ID NO:61), linked to KLH (*see* Newton et al., *Dev. Dynamics 197*:1-13, 1993). To evaluate the specificity of a particular antibody, representative assays as described herein and/or conventional antigen-binding assays may be employed. Such antibodies may generally be used for therapeutic, diagnostic and assay purposes, as described herein. For example, such antibodies may be linked to a drug and administered to a mammal to target the drug to a particular cadherin-expressing cell, such as a leukemic cell in the blood.

Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane,

Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988; *see* especially page 309) and digested by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by affinity chromatography on protein A bead columns (Harlow and Lane, 1988, pages 628-29).

EVALUATION OF MODULATING AGENT ACTIVITY

As noted above, cyclic peptides and other modulating agents as described herein are capable of modulating (*i.e.*, enhancing or inhibiting) cadherin-mediated cell adhesion. The ability of a modulating agent to modulate cell adhesion may generally be evaluated *in vitro* by assaying the effect of the cyclic peptide on one or more of the following: (1) neurite outgrowth, (2) adhesion between endothelial cells, (3) adhesion between epithelial cells (*e.g.*, normal rat kidney cells and/or human skin) and/or (4) adhesion between cancer cells. In general, a modulating agent is an inhibitor of cell adhesion if, within one or more of these representative assays, contact of the test cells with the modulating agent results in a discernible disruption of cell adhesion. Modulating agents that enhance cell adhesion (*e.g.*, agents comprising multiple HAV sequences and/or linked to a support material) are considered to be modulators of cell adhesion if they are capable of enhancing neurite outgrowth as described below and/or are capable of promoting cell adhesion, as judged by plating assays to assess epithelial cell adhesion to a modulating agent attached to a support material, such as tissue culture plastic.

Within a representative neurite outgrowth assay, neurons may be cultured on a monolayer of cells (*e.g.*, 3T3) that express N-cadherin. Neurons grown on such cells (under suitable conditions and for a sufficient period of time) extend longer neurites than neurons cultured on cells that do not express N-cadherin. For example, neurons may be cultured on monolayers of 3T3 cells transfected with cDNA encoding N-cadherin essentially as described by Doherty and Walsh, *Curr. Op. Neurobiol.* *4*:49-55, 1994; Williams et al., *Neuron* *13*:583-594, 1994; Hall et al., *Cell Adhesion and Commun.* *3*:441-450, 1996; Doherty and Walsh, *Mol. Cell. Neurosci.* *8*:99-111, 1994; and Safell et al., *Neuron* *18*:231-242, 1997. Briefly, monolayers of control 3T3

fibroblasts and 3T3 fibroblasts that express N-cadherin may be established by overnight culture of 80,000 cells in individual wells of an 8-chamber well tissue culture slide. 3000 cerebellar neurons isolated from post-natal day 3 mouse brains may be cultured for 18 hours on the various monolayers in control media (SATO/2%FCS), or media supplemented with various concentrations of the modulating agent or control peptide. The cultures may then be fixed and stained for GAP43 which specifically binds to the neurons and their neurites. The length of the longest neurite on each GAP43 positive neuron may be measured by computer assisted morphometry.

A modulating agent that modulates N-cadherin-mediated cell adhesion may inhibit or enhance such neurite outgrowth. Under the conditions described above, the presence of 500 µg/mL of a modulating agent that disrupts neural cell adhesion should result in a decrease in the mean neurite length by at least 50%, relative to the length in the absence of modulating agent or in the presence of a negative control peptide. Alternatively, the presence of 500 µg/mL of a modulating agent that enhances neural cell adhesion should result in an increase in the mean neurite length by at least 50%.

Within one representative cell adhesion assay, the addition of a modulating agent to cells that express a cadherin results in disruption of cell adhesion. A "cadherin-expressing cell," as used herein, may be any type of cell that expresses at least one cadherin on the cell surface at a detectable level, using standard techniques such as immunocytochemical protocols (Blaschuk and Farookhim, *Dev. Biol. 136*:564-567, 1989). Cadherin-expressing cells include endothelial (*e.g.*, bovine pulmonary artery endothelial cells), epithelial and/or cancer cells (*e.g.*, the human ovarian cancer cell line SKOV3 (ATCC #HTB-77)). For example, such cells may be plated under standard conditions that permit cell adhesion in the presence and absence of modulating agent (*e.g.*, 500 µg/mL). Disruption of cell adhesion may be determined visually within 24 hours, by observing retraction of the cells from one another.

For use within one such assay, bovine pulmonary artery endothelial cells may be harvested by sterile ablation and digestion in 0.1% collagenase (type II; Worthington Enzymes, Freehold, NJ). Cells may be maintained in Dulbecco's

minimum essential medium supplemented with 10% fetal calf serum and 1% antibiotic-antimycotic at 37°C in 7% CO_2 in air. Cultures may be passaged weekly in trypsin-EDTA and seeded onto tissue culture plastic at 20,000 cells/cm². Endothelial cultures may be used at 1 week in culture, which is approximately 3 days after culture confluency is established. The cells may be seeded onto coverslips and treated (*e.g.*, for 30 minutes) with modulating agent or a control compound at, for example, 500µg/ml and then fixed with 1% paraformaldehyde. As noted above, disruption of cell adhesion may be determined visually within 24 hours, by observing retraction of the cells from one another. This assay evaluates the effect of a modulating agent on N-cadherin mediated cell adhesion.

Within another such assay, the effect of a modulating agent on normal rat kidney (NRK) cells may be evaluated. According to a representative procedure, NRK cells (ATCC #1571-CRL) may be plated at 10 – 20,000 cells per 35mm tissue culture flasks containing DMEM with 10% FCS and sub-cultured periodically (Laird et al., *J. Cell Biol. 131*:1193-1203, 1995). Cells may be harvested and replated in 35mm tissue culture flasks containing 1 mm coverslips and incubated until 50–65% confluent (24-36 hours). At this time, coverslips may be transferred to a 24-well plate, washed once with fresh DMEM and exposed to modulating agent at a concentration of, for example, 1mg/mL for 24 hours. Fresh modulating agent may then be added, and the cells left for an additional 24 hours. Cells may be fixed with 100% methanol for 10 minutes and then washed three times with PBS. Coverslips may be blocked for 1 hour in 2% BSA/PBS and incubated for a further 1 hour in the presence of mouse anti-E-cadherin antibody (Transduction Labs, 1:250 dilution). Primary and secondary antibodies may be diluted in 2% BSA/PBS. Following incubation in the primary antibody, coverslips may be washed three times for 5 minutes each in PBS and incubated for 1 hour with donkey anti-mouse antibody conjugated to fluorescein (diluted 1:200). Following further washes in PBS (3 x 5 min) coverslips can be mounted and viewed by confocal microscopy.

In the absence of modulating agent, NRK cells form characteristic tightly adherent monolayers with a cobblestone morphology in which cells display a polygonal

shape. NRK cells that are treated with a modulating agent that disrupts E-cadherin mediated cell adhesion may assume a non-polygonal and elongated morphology (*i.e.*, a fibroblast-like shape) within 48 hours of treatment with 1 mg/mL of modulating agent. Gaps appear in confluent cultures of such cells. In addition, 1 mg/mL of such a modulating agent reproducibly induces a readily apparent reduction in cell surface staining of E-cadherin, as judged by immunofluorescence microscopy (Laird et al., *J. Cell Biol.* *131*:1193-1203, 1995), of at least 75% within 48 hours.

A third cell adhesion assay involves evaluating the effect of a cyclic peptide on permeability of adherent epithelial and/or endothelial cell layers. For example, the effect of permeability on human skin may be evaluated. Such skin may be derived from a natural source or may be synthetic. Human abdominal skin for use in such assays may generally be obtained from humans at autopsy within 24 hours of death. Briefly, a cyclic peptide and a test marker (*e.g.*, the fluorescent markers Oregon Green™ and Rhodamine Green™ Dextran) may be dissolved in a sterile buffer, and the ability of the marker to penetrate through the skin and into a receptor fluid may be measured using a Franz Cell apparatus (Franz, *Curr. Prob. Dermatol.* 7:58-68, 1978; Franz, *J. Invest. Dermatol.* *64*:190-195, 1975). In general, a modulating agent that enhances the permeability of human skin results in a statistically significant increase in the amount of marker in the receptor compartment after 6-48 hours in the presence of 500 µg/mL modulating agent. This assay evaluates the effect of a modulating agent on E-cadherin mediated cell adhesion.

MODULATING AGENT MODIFICATION AND FORMULATIONS

A modulating agent as described herein may, but need not, be linked to one or more additional molecules. In particular, as discussed below, it may be beneficial for certain applications to link multiple modulating agents (which may, but need not, be identical) to a support molecule (*e.g.*, keyhole limpet hemocyanin) or a solid support, such as a polymeric matrix (which may be formulated as a membrane or microstructure, such as an ultra thin film), a container surface (*e.g.*, the surface of a tissue culture plate or the interior surface of a bioreactor), or a bead or other particle,

which may be prepared from a variety of materials including glass, plastic or ceramics. For certain applications, biodegradable support materials are preferred, such as cellulose and derivatives thereof, collagen, spider silk or any of a variety of polyesters (*e.g.*, those derived from hydroxy acids and/or lactones) or sutures (*see* U.S. Patent No. 5,245,012). Within certain embodiments, modulating agents and molecules comprising other CAR sequence(s) (*e.g.*, an RGD and/or LYHY (SEQ ID NO:58) sequence) may be attached to a support such as a polymeric matrix, preferably in an alternating pattern.

Suitable methods for linking a modulating agent to a support material will depend upon the composition of the support and the intended use, and will be readily apparent to those of ordinary skill in the art. Attachment may generally be achieved through noncovalent association, such as adsorption or affinity or, preferably, via covalent attachment (which may be a direct linkage between a modulating agent and functional groups on the support, or may be a linkage by way of a cross-linking agent or linker). Attachment of a modulating agent by adsorption may be achieved by contact, in a suitable buffer, with a solid support for a suitable amount of time. The contact time varies with temperature, but is generally between about 5 seconds and 1 day, and typically between about 10 seconds and 1 hour.

Covalent attachment of a modulating agent to a molecule or solid support may generally be achieved by first reacting the support material with a bifunctional reagent that will also react with a functional group, such as a hydroxyl, thiol, carboxyl, ketone or amino group, on the modulating agent. For example, a modulating agent may be bound to an appropriate polymeric support or coating using benzoquinone, by condensation of an aldehyde group on the support with an amine and an active hydrogen on the modulating agent or by condensation of an amino group on the support with a carboxylic acid on the modulating agent. A preferred method of generating a linkage is via amino groups using glutaraldehyde. A modulating agent may be linked to cellulose via ester linkages. Similarly, amide linkages may be suitable for linkage to other molecules such as keyhole limpet hemocyanin or other support materials. Multiple modulating agents and/or molecules comprising other CAR sequences may be attached, for example, by random coupling, in which equimolar

amounts of such molecules are mixed with a matrix support and allowed to couple at random.

Although modulating agents as described herein may preferentially bind to specific tissues or cells, and thus may be sufficient to target a desired site *in vivo*, it may be beneficial for certain applications to include an additional targeting agent. Accordingly, a targeting agent may also, or alternatively, be linked to a modulating agent to facilitate targeting to one or more specific tissues. As used herein, a "targeting agent," may be any substance (such as a compound or cell) that, when linked to a modulating agent enhances the transport of the modulating agent to a target tissue, thereby increasing the local concentration of the modulating agent. Targeting agents include antibodies or fragments thereof, receptors, ligands and other molecules that bind to cells of, or in the vicinity of, the target tissue. Known targeting agents include serum hormones, antibodies against cell surface antigens, lectins, adhesion molecules, tumor cell surface binding ligands, steroids, cholesterol, lymphokines, fibrinolytic enzymes and those drugs and proteins that bind to a desired target site. Among the many monoclonal antibodies that may serve as targeting agents are anti-TAC, or other interleukin-2 receptor antibodies; 9.2.27 and NR-ML-05, reactive with the 250 kilodalton human melanoma-associated proteoglycan; and NR-LU-10, reactive with a pancarcinoma glycoprotein. An antibody targeting agent may be an intact (whole) molecule, a fragment thereof, or a functional equivalent thereof. Examples of antibody fragments are F(ab')2, -Fab', Fab and F[v] fragments, which may be produced by conventional methods or by genetic or protein engineering. Linkage is generally covalent and may be achieved by, for example, direct condensation or other reactions, or by way of bi- or multi-functional linkers. Within other embodiments, it may also be possible to target a polynucleotide encoding a modulating agent to a target tissue, thereby increasing the local concentration of modulating agent. Such targeting may be achieved using well known techniques, including retroviral and adenoviral infection.

For certain embodiments, it may be beneficial to also, or alternatively, link a drug to a modulating agent. As used herein, the term "drug" refers to any bioactive agent intended for administration to a mammal to prevent or treat a disease or

other undesirable condition. Drugs include hormones, growth factors, proteins, peptides and other compounds. The use of certain specific drugs within the context of the present invention is discussed below.

Within certain aspects of the present invention, one or more modulating agents as described herein may be present within a pharmaceutical composition. A pharmaceutical composition comprises one or more modulating agents in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide) and/or preservatives. Within yet other embodiments, compositions of the present invention may be formulated as a lyophilizate. A modulating agent (alone or in combination with a targeting agent and/or drug) may, but need not, be encapsulated within liposomes using well known technology. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous, or intramuscular administration. For certain topical applications, formulation as a cream or lotion, using well known components, is preferred.

For certain embodiments, as discussed below, a pharmaceutical composition may further comprise a modulator of cell adhesion that is mediated by one or more molecules other than cadherins. Such modulators may generally be prepared as described above, incorporating one or more non-cadherin CAR sequences and/or antibodies thereto in place of the cadherin CAR sequences and antibodies. Such compositions are particularly useful for situations in which it is desirable to inhibit cell adhesion mediated by multiple cell-adhesion molecules, such as other members of the cadherin gene superfamily that are not classical cadherins (*e.g.*, Dsg and Dsc); integrins; members of the immunoglobulin supergene family, such as N-CAM; and other uncategorized transmembrane proteins, such as occludin, as well as extracellular matrix proteins such as laminin, fibronectin, collagens, vitronectin, entactin and tenascin.

Preferred CAR sequences for use within such a modulator include RGD, YIGSR (SEQ ID NO:47), KYSFNYDGSE (SEQ ID NO:62), IWKHKGRDVILKKDVRF (SEQ ID NO:63), YAT, FAT, YAS, RAL and/or GVNPTAQSSGSLYGSQIYALCNQFYTPAATGLYVDQYLYHYCVVDPQE (SEQ ID NO:59), or derivatives or portions thereof such as QYLYHYCVVD (SEQ ID NO:60) and LYHY (SEQ ID NO:58).

A pharmaceutical composition may also contain one or more drugs, which may be linked to a modulating agent or may be free within the composition. Virtually any drug may be administered in combination with a cyclic peptide as described herein, for a variety of purposes as described below. Examples of types of drugs that may be administered with a cyclic peptide include analgesics, anesthetics, antianginals, antifungals, antibiotics, anticancer drugs (*e.g.*, taxol or mitomycin C), antiinflammatories (*e.g.*, ibuprofen and indomethacin), anthelmintics, antidepressants, antidotes, antiemetics, antihistamines, antihypertensives, antimalarials, antimicrotubule agents (*e.g.*, colchicine or vinca alkaloids), antimigraine agents, antimicrobials, antiphsychotics, antipyretics, antiseptics, anti-signaling agents (*e.g.*, protein kinase C inhibitors or inhibitors of intracellular calcium mobilization), antiarthritics, antithrombin agents, antituberculotics, antitussives, antivirals, appetite suppressants, cardioactive drugs, chemical dependency drugs, cathartics, chemotherapeutic agents, coronary, cerebral or peripheral vasodilators, contraceptive agents, depressants, diuretics, expectorants, growth factors, hormonal agents, hypnotics, immunosuppression agents, narcotic antagonists, parasympathomimetics, sedatives, stimulants, sympathomimetics, toxins (*e.g.*, cholera toxin), tranquilizers and urinary antiinfectives.

For imaging purposes, any of a variety of diagnostic agents may be incorporated into a pharmaceutical composition, either linked to a modulating agent or free within the composition. Diagnostic agents include any substance administered to illuminate a physiological function within a patient, while leaving other physiological functions generally unaffected. Diagnostic agents include metals, radioactive isotopes and radioopaque agents (*e.g.*, gallium, technetium, indium, strontium, iodine, barium,

bromine and phosphorus-containing compounds), radiolucent agents, contrast agents, dyes (*e.g.*, fluorescent dyes and chromophores) and enzymes that catalyze a colorimetric or fluorometric reaction. In general, such agents may be attached using a variety of techniques as described above, and may be present in any orientation.

The compositions described herein may be administered as part of a sustained release formulation (*i.e.*, a formulation such as a capsule or sponge that effects a slow release of cyclic peptide following administration). Such formulations may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a cyclic peptide dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane (*see, e.g.*, European Patent Application 710,491 A). Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of cyclic peptide release. The amount of cyclic peptide contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented). Appropriate dosages and the duration and frequency of administration will be determined by such factors as the condition of the patient, the type and severity of the patient's disease and the method of administration. In general, an appropriate dosage and treatment regimen provides the modulating agent(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Within particularly preferred embodiments of the invention, a modulating agent or pharmaceutical composition as described herein may be administered at a dosage ranging from 0.001 to 50 mg/kg body weight, preferably from 0.1 to 20 mg/kg, on a regimen of single or multiple daily doses. For topical administration, a cream typically comprises an amount of modulating agent ranging from 0.00001% to 1%, preferably 0.0001% to 0.2%, and more preferably from 0.0001% to 0.002%. Fluid compositions typically contain about 10 ng/ml to 5 mg/ml,

preferably from about 10 μg to 2 mg/mL cyclic peptide. Appropriate dosages may generally be determined using experimental models and/or clinical trials. In general, the use of the minimum dosage that is sufficient to provide effective therapy is preferred. Patients may generally be monitored for therapeutic effectiveness using assays suitable for the condition being treated or prevented, which will be familiar to those of ordinary skill in the art.

MODULATING AGENT METHODS OF USE

In general, the modulating agents and compositions described herein may be used for modulating the adhesion of cadherin-expressing cells (*i.e.*, cells that express one or more of E-cadherin, N-cadherin, P-cadherin, R-cadherin and/or other cadherin(s) containing the HAV sequence, including as yet undiscovered cadherins) *in vitro* and/or *in vivo*. As noted above, modulating agents for purposes that involve the disruption of cadherin-mediated cell adhesion may comprise a cyclic peptide containing a single HAV sequence, multiple HAV sequences in close proximity and/or an antibody (or an antigen-binding fragment thereof) that recognizes a cadherin CAR sequence. When it is desirable to also disrupt cell adhesion mediated by other adhesion molecules, a modulating agent may additionally comprise one or more CAR sequences bound by such adhesion molecules (and/or antibodies or fragments thereof that bind such sequences), preferably separated from each other and from the HAV sequence by linkers. As noted above, such linkers may or may not comprise one or more amino acids. For enhancing cell adhesion, a modulating agent may contain multiple HAV sequences or antibodies (or fragments), preferably separated by linkers, and/or may be linked to a single molecule or to a support material as described above.

Certain methods involving the disruption of cell adhesion as described herein have an advantage over prior techniques in that they permit the passage of molecules that are large and/or charged across barriers of cadherin-expressing cells. As discussed in greater detail below, modulating agents as described herein may also be used to disrupt or enhance cell adhesion in a variety of other contexts. Within the methods described herein, one or more modulating agents may generally be

administered alone, or within a pharmaceutical composition. In each specific method described herein, as noted above, a targeting agent may be employed to increase the local concentration of modulating agent at the target site.

In one such aspect, the present invention provides methods for reducing unwanted cellular adhesion by administering a cyclic peptide as described herein. Unwanted cellular adhesion can occur between tumor cells, between tumor cells and normal cells or between normal cells as a result of surgery, injury, chemotherapy, disease, inflammation or other condition jeopardizing cell viability or function. Preferred modulating agents for use within such methods comprise a cyclic peptide such as N-Ac-CHAVC-NH_2 (SEQ ID NO:8), N-Ac-CHAVDC-NH_2 (SEQ ID NO:48), N-Ac-CAHAVC-NH_2 (SEQ ID NO:49), N-Ac-CAHAVDC-NH_2 (SEQ ID NO:16), N-Ac-CAHAVDIC-NH_2 (SEQ ID NO:10), N-Ac-CRAHAVDC-NH_2 (SEQ ID NO:50), N-Ac-CLRAHAVC-NH_2 (SEQ ID NO:51), N-Ac-CLRAHAVDC-NH_2 (SEQ ID NO:52), N-Ac-CSHAVC-NH_2 (SEQ ID NO:12), N-Ac-CHAVSC-NH_2 (SEQ ID NO:14), N-Ac-CSHAVSC-NH_2 (SEQ ID NO:53), N-Ac-CSHAVSSC-NH_2 (SEQ ID NO:18), N-Ac-CHAVSSC-NH_2 (SEQ ID NO:54), N-Ac-KHAVD-NH_2 (SEQ ID NO:20), N-Ac-DHAVK-NH_2 (SEQ ID NO:55), N-Ac-KHAVE-NH_2 (SEQ ID NO:56), N-Ac-AHAVDI-NH_2 (SEQ ID NO:44), N-Ac-SHAVDSS-NH_2 (SEQ ID NO:57), N-Ac-KSHAVSSD-NH_2 (SEQ ID NO:45) and derivatives thereof, including derivatives without the N-acetyl group. In addition, a modulating agent may comprise the sequence RGD, which is bound by integrins, and/or the sequence LYHY (SEQ ID NO:58), which is bound by occludin, separated from the HAV sequence via a linker. Alternatively, a separate modulator of integrin- and/or occludin-mediated cell adhesion may be administered in conjunction with the modulating agent(s), either within the same pharmaceutical composition or separately. Topical administration of the modulating agent(s) is generally preferred, but other means may also be employed. Preferably, a fluid composition for topical administration (comprising, for example, physiological saline) comprises an amount of cyclic peptide as described above, and more preferably an amount ranging from 10μg/mL to 1mg/mL. Creams may generally be formulated as described above. Topical administration in the surgical field may be given once at the

end of surgery by irrigation of the wound, as an intermittent or continuous irrigation with use of surgical drains in the post operative period, or by the use of drains specifically inserted in an area of inflammation, injury or disease in cases where surgery does not need to be performed. Alternatively, parenteral or transcutaneous administration may be used to achieve similar results.

Within a further aspect, modulating agents as described herein may be used for controlled inhibition of synaptic stability, resulting in increased synaptic plasticity. Within this aspect, administration of one or more modulating agents may be advantageous for repair processes within the brain, as well as learning and memory, in which neural plasticity is a key early event in the remodeling of synapses. Cell adhesion molecules, particularly N-cadherin and E-cadherin, can function to stabilize synapses, and loss of this function is thought to be the initial step in the remodeling of the synapse that is associated with learning and memory (Doherty et al., *J. Neurobiology, 26*:437-446, 1995; Martin and Kandel, *Neuron, 17*:567-570, 1996; Fannon and Colman, *Neuron, 17*:423-434, 1996). Inhibition of cadherin function by administration of one or more modulating agents that inhibit cadherin function may stimulate learning and memory.

Preferred modulating agents for use within such methods include those that disrupt E-cadherin and/or N-cadherin mediated cell adhesion, and comprise cyclic peptides such as N-Ac-<u>CHAVC</u>-NH_2 (SEQ ID NO:8), N-Ac-<u>CHAVDC</u>-NH_2 (SEQ ID NO:48), N-Ac-<u>CAHAVC</u>-NH_2 (SEQ ID NO:49), N-Ac-<u>CAHAVDC</u>-NH_2 (SEQ ID NO:16), N-Ac-<u>CAHAVDIC</u>-NH_2 (SEQ ID NO:10), N-Ac-<u>CRAHAVDC</u>-NH_2 (SEQ ID NO:50), N-Ac-<u>CLRAHAVC</u>-NH_2 (SEQ ID NO:51), N-Ac-<u>CLRAHAVDC</u>-NH_2 (SEQ ID NO:52), N-Ac-<u>CSHAVC</u>-NH_2 (SEQ ID NO:12), N-Ac-<u>CHAVSC</u>-NH_2 (SEQ ID NO:14), N-Ac-<u>CSHAVSC</u>-NH_2 (SEQ ID NO:53), N-Ac-<u>CSHAVSSC</u>-NH_2 (SEQ ID NO:18), N-Ac-<u>CHAVSSC</u>-NH_2 (SEQ ID NO:54), N-Ac-<u>KHAVD</u>-NH_2 (SEQ ID NO:20), N-Ac-<u>DHAVK</u>-NH_2 (SEQ ID NO:55), N-Ac-<u>KHAVE</u>-NH_2 (SEQ ID NO:56), N-Ac-<u>AHAVDI</u>-NH_2 (SEQ ID NO:44), N-Ac-<u>SHAVDSS</u>-NH_2 (SEQ ID NO:57), N-Ac-<u>KSHAVSSD</u>-NH_2 (SEQ ID NO:45) and derivatives thereof, including derivatives without the N-acetyl group. In addition, a preferred modulating agent may comprise

one or more additional CAR sequences, such as the sequence RGD, which is bound by integrins and/or the N-CAM CAR sequence KYSFNYDGSE (SEQ ID NO:62). As noted above, such additional sequence(s) may be separated from the HAV sequence via a linker. Alternatively, a separate modulator of integrin and/or N-CAM mediated cell adhesion may be administered in conjunction with the modulating agent(s), either within the same pharmaceutical composition or separately. For such aspects, administration may be via encapsulation into a delivery vehicle such as a liposome, using standard techniques, and injection into, for example, the carotid artery. Alternatively, a modulating agent may be linked to a disrupter of the blood-brain barrier. In general dosages range as described above.

Other aspects of the present invention provide methods that employ antibodies raised against the modulating agents described above. Such polyclonal and monoclonal antibodies may be raised against a cyclic peptide using conventional techniques known to those of ordinary skill in the art. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In one such technique, an immunogen comprising the cyclic peptide is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep or goats). Because of its small size, the cyclic peptide should be joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. Following one or more injections, the animals are bled periodically. Polyclonal antibodies specific for the cyclic peptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for the cyclic peptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* *6*:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity from spleen cells obtained from an animal immunized as described above. The spleen cells are immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. Single colonies

are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies, with or without the use of various techniques known in the art to enhance the yield. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. Antibodies having the desired activity may generally be identified using immunofluorescence analyses of tissue sections, cell or other samples where the target cadherin is localized.

Cyclic peptides may also be used to generate monoclonal antibodies, as described above, that are specific for particular cadherins (*e.g.*, antibodies that bind to E-cadherin, but do not bind significantly to N-cadherin, or vise versa). Such antibodies may generally be used *in vitro* or *in vivo* to modulate cell adhesion. Within certain embodiments, antibodies (or, preferably, antigen-binding fragments thereof) may be used for controlled inhibition of synaptic stability, as described above. The use of Fab fragments is generally preferred.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

Preparation of Representative Cyclic Peptides

This Example illustrates the solid phase synthesis of representative cyclic peptides.

The peptides were assembled on methylbenzhydrylamine resin (MBHA resin) for the C-terminal amide peptides. The traditional Merrifield resins were used for any C-terminal acid peptides. Bags of a polypropylene mesh material were filled with the resin and soaked in dichloromethane. The resin packets were washed three times with 5% diisopropylethylamine in dichloromethane and then washed with dichloromethane. The packets are then sorted and placed into a Nalgene bottle containing a solution of the amino acid of interest in dichloromethane. An equal amount of diisopropylcarbodiimide (DIC) in dichloromethane was added to activate the coupling reaction. The bottle was shaken for one hour to ensure completion of the reaction. The reaction mixture was discarded and the packets washed with DMF. The N-α-Boc was removed by acidolysis using a 55% TFA in dichloromethane for 30 minutes leaving the TFA salt of the α-amino group. The bags were washed and the synthesis completed by repeating the same procedure while substituting for the corresponding amino acid at the coupling step. Acetylation of the N-terminal was performed by reacting the peptide resins with a solution of acetic anhydride in dichloromethane in the presence of diisopropylethylamine. The peptide was then side-chain deprotected and cleaved from the resin at 0°C with liquid HF in the presence of anisole as a carbocation scavenger.

The crude peptides were purified by reversed-phase high-performance liquid chromatography. Purified linear precursors of the cyclic peptides were solubilized in 75% acetic acid at a concentration of 2-10mg/mL. A 10% solution of iodine in methanol was added dropwise until a persistent coloration was obtained. A 5% ascorbic acid solution in water was then added to the mixture until discoloration. The disulfide bridge containing compounds were then purified by HPLC and characterized by analytical HPLC and by mass spectral analysis.

EXAMPLE 2

Disruption of the Ability of Mouse Cerebellar Neurons to Extend Neurites

Three cell adhesion molecules, N-cadherin, N-CAM and L1, are capable of regulating neurite outgrowth (Doherty and Walsh, *Curr. Op. Neurobiol. 4*:49-55, 1994; Williams et al., *Neuron 13*:583-594, 1994; Hall et al., *Cell Adhesion and Commun. 3*:441-450, 1996; Doherty and Walsh, *Mol. Cell. Neurosci. 8*:99-111, 1994; Safell et al., *Neuron 18*:231-242, 1997). Neurons cultured on monolayers of 3T3 cells that have been transfected with cDNAs encoding N-cadherin, N-CAM or L1 extend longer neurites than neurons cultured on 3T3 cells not expressing these cell adhesion molecules. This Example illustrates the use of a representative cyclic peptide to inhibit neurite outgrowth.

Neurons were cultured on monolayers of 3T3 cells transfected with cDNA encoding N-cadherin essentially as described by Doherty and Walsh, *Curr. Op. Neurobiol. 4*:49-55, 1994; Williams et al., *Neuron 13*:583-594, 1994; Hall et al., *Cell Adhesion and Commun. 3*:441-450, 1996; Doherty and Walsh, *Mol. Cell. Neurosci.* 8:99-111, 1994; Safell et al., *Neuron 18*:231-242, 1997. Briefly, monolayers of control 3T3 fibroblasts and 3T3 fibroblasts that express N-cadherin were established by overnight culture of 80,000 cells in individual wells of an 8-chamber well tissue culture slide. 3000 cerebellar neurons isolated from post-natal day 3 mouse brains were cultured for 18 hours on the various monolayers in control media (SATO/2%FCS), or media supplemented with various concentrations of the cyclic peptide N-Ac-<u>CHAVC</u>-NH_2 or a control peptide without the HAV sequence (N-Ac-<u>CHGVC</u>-NH_2). The cultures were then fixed and stained for GAP43 which specifically binds to the neurons and their neurites. The length of the longest neurite on each GAP43 positive neuron was then measured by computer assisted morphometry.

As shown in Figure 4, culture for 18 hours with N-Ac-<u>CHAVC</u>-NH_2 at a concentration of 500 μg/mL inhibited neurite outgrowth on 3T3 cells expressing N-cadherin, whereas the cyclic peptide N-Ac-<u>CHGVC</u>-NH_2 (also at a concentration of 500 μg/ml) had no effect on this process. Furthermore, the cyclic peptide N-Ac-<u>CHAVC</u>-

NH_2 (used at a concentration of 500 μg/ml) did not inhibit neurite outgrowth on 3T3 cells not expressing N-cadherin, N-CAM, or L1 (control cells), thus indicating that the peptide is not toxic and that it has no non-specific effects on neurite outgrowth (Figure 4, compare columns 3 and 1). These data also indicate that the peptide does not effect integrin function.

A dose-response study demonstrated that N-Ac-<u>CHAVC</u>-NH_2 significantly inhibited neurite outgrowth on 3T3 cells expressing N-cadherin at a concentration of 50 μg/mL, and completely inhibited neurite outgrowth on these cells at a concentration of 500 μg/mL (Figure 5). Finally, N-Ac-<u>CHAVC</u>-NH_2 (used at a concentration of 500 μg/mL) did not inhibit neurite outgrowth on 3T3 cells expressing either N-CAM or L1 (Figure 6). These results indicate that the peptide N-Ac-<u>CHAVC</u>-NH_2 specifically inhibits the function of N-cadherin. Collectively, the results obtained from these studies demonstrate that N-Ac-<u>CHAVC</u>-NH_2 is an effective inhibitor of neurite outgrowth by virtue of its ability to disrupt N-cadherin function.

EXAMPLE 3

<u>Disruption of Bovine Endothelial Cell Adhesion</u>

This Example illustrates the use of representative cyclic peptides to disrupt adhesion of endothelial cells, which express N-cadherin.

Bovine pulmonary artery endothelial cells were harvested by sterile ablation and digestion in 0.1% collagenase (type II; Worthington Enzymes, Freehold, NJ). Cells were maintained in Dulbecco's minimum essential medium (Clonetics, San Diego, CA) supplemented with 10% fetal calf serum (Atlantic Biologicals, Nor cross, GA) and 1% antibiotic-antimycotic at 37°C in 7% CO_2 in air. Cultures were passaged weekly in trypsin-EDTA (Gibco, Grand Island, NY) and seeded onto tissue culture plastic at 20,000 cells/cm^2 for all experiments. Endothelial cultures were used at 1 week in culture, which is approximately 3 days after culture confluency was established. The cells used in all protocols were between 4th passage and 10th passage. The cells were

seeded onto coverslips and treated 30 minutes with N-Ac-CHAVC-NH_2 or N-Ac-CHGVC-NH_2 at 500µg/ml and then fixed with 1% paraformaldehyde.

The peptide N-Ac-CHAVC-NH_2 disrupted the endothelial cell monolayer within 30 minutes after being added to the culture medium, whereas N-Ac-CHGVC-NH_2 had no affect on the cells (Figure 7). Endothelial cell morphology was dramatically affected by N-Ac-CHAVC-NH_2, and the cells retracted from one another and became non-adherent. These data demonstrate that N-Ac-CHAVC-NH_2 is capable of inhibiting endothelial cell adhesion.

Under the same conditions, the cyclic peptides H-CHAVC-NH_2, N-Ac-CAHAVDIC-NH_2 (SEQ ID NO:10) (Figure 8) and N-Ac-CHAVSC-NH_2 (SEQ ID NO:14) had no effect on endothelial cell morphology, indicating that not all cyclic HAV-containing peptides are capable of disrupting endothelial cell adhesion at a concentration of 500µg/mL. It is not unexpected that the potencies of individual cyclic peptides will vary The cyclic peptide N-Ac-CAHAVDC-NH_2 (SEQ ID NO:16; Figure 9) had a slight effect while N-Ac-CSHAVSSC-NH_2 (SEQ ID NO:18; Figure 10) disrupted the endothelial cell monolayer and caused the cells to retract from one another.

EXAMPLE 4

Disruption of Human Ovarian Cancer Cell Adhesion

This Example illustrates the use of a representative cyclic peptide to disrupt adhesion of human ovarian cancer cells.

The human ovarian cancer cell line SKOV3 (ATCC #HTB-77) expresses N-cadherin. SKOV3 cells were cultured in a modified MEM-based media containing 10% FCS. Cells were grown in T-250 culture flasks and maintained by periodic subculturing. Cyclic peptides were tested on cells grown in individual wells of 96-well culture dishes (surface area of each well was 0.32cm^2). Cells were harvested from flasks and seeded at a density of 50,000 cells per well in 0.1mL media containing the cyclic peptides at concentrations of 1, 0.1, or 0.01 mg/mL, or in the absence of cyclic

peptide. Media control wells were also established. Cultures were evaluated periodically by microscopic examination under both bright field and phase contrast conditions. Cultures were maintained for 48 hours.

As shown in Figures 11A (compare to Figure 11C) and 12A, the peptide N-Ac-CHAVC-NH_2 (final concentration of 1 mg/mL media) disrupted SKOV3 cell adhesion within 24 hours, whereas the control N-Ac-CHGVC-NH_2 had no affect on cell adhesion (Figures 11B and 12B). The effect of different amounts of N-Ac-CHAVC-NH_2 after 48 hours is shown in Figures 11D-F. In the presence of N-Ac-CHGVC-NH_2, (Figures 11B and 12B) the SKOV3 cells formed tightly adherent monolayers. In contrast, the SKOV3 cells did not spread onto the substrata, nor did they form tightly adherent monolayers in the presence of N-Ac-CHAVC-NH_2 (Figures 11A, D and 12A). These data demonstrate that N-Ac-CHAVC-NH_2 is capable of inhibiting the function of human N-cadherin.

The cyclic peptides N-Ac-CAHAVDIC-NH_2, N-Ac-CAHAVDC-NH_2 (SEQ ID NO:16) and N-Ac-KHAVD-NH_2 (SEQ ID NO:20) were inactive in the SKOV3 cells, indicating that not all cyclic HAV-containing peptides are capable of disrupting epithelial cell adhesion at concentrations of 0.01-1 mg/mL It is not unexpected that the potencies of the cyclic peptides will vary.

EXAMPLE 5

Disruption of Angiogenesis

Blood vessels are composed of adherent endothelial cells. This Example illustrates the use of a representative cyclic peptide to block angiogenesis (the growth of blood vessels from pre-existing blood vessels).

The chick chorioallantoic membrane assay was used to assess the effects of cyclic peptides on angiogenesis (Iruela-Arispe et al., *Molecular Biology of the Cell* 6:327-343, 1995). Cyclic peptides were embedded in a mesh composed of vitrogen at concentrations of 3, 17, and 33 μg/mesh. The meshes were then applied to 12-day-old

chick embryonic chorioallantoic membranes. After 24 hours, the effects of the peptides on angiogenesis were assessed by computer assisted morphometric analysis.

The ability of representative cyclic peptides to inhibit angiogenesis is illustrated by the results presented in Table 2. For each concentration of cyclic peptide, the percent inhibition of angiogenesis (relative to the level of angiogenesis in the absence of cyclic peptide) is provided. Assays were performed in the presence (+) or absence (-) of 0.01mM VEGF. For example, the cyclic peptide N-Ac-<u>CHAVC</u>-NH_2 (SEQ ID NO:8) inhibited angiogenesis by 46%, 51%, and 51% at concentrations of 3, 17, and 33 µg/mesh, respectively. The N-cadherin selective peptides N-Ac-<u>CAHAVDIC</u>-NH_2 (SEQ ID NO:10) and N-Ac-<u>CAHAVDC</u>-NH_2 (SEQ ID NO:16) also inhibited angiogenesis significantly. The E-cadherin selective cyclic peptides N-Ac-<u>CHAVSC</u>-NH_2 (SEQ ID NO:14) and N-Ac-<u>CSHAVSSC</u>-NH_2 (SEQ ID NO:18), as well as the scrambled peptide N-Ac-<u>CVAHC</u>-NH_2, were found to be relatively inactive in this assay.

<u>Table 2</u>

Compound	Concentration, µg / mesh ± VEGF					
	3(-)	3(+)	17(-)	17(+)	33(-)	33(+)
H-<u>CHAVC</u>-NH_2	11%	27%	13%	34%	17%	35%
N-Ac-<u>CHAVSC</u>-NH_2	11%	17%	12%	16%	17%	19%
N-Ac-<u>CVAHC</u>-NH_2	-1%	7%	13%	24%	12%	25%
N-Ac-<u>CHAVC</u>-NH_2	12%	46%	22%	51%	28%	51%
N-Ac-<u>CAHAVDIC</u>-NH_2	-1%	21%	15%	37%	33%	49%
N-Ac-<u>CAHAVDC</u>-NH_2	21%	59%	27%	72%	31%	79%
N-Ac-<u>CSHAVSSC</u>-NH_2	1%	-3%	-3%	12%	17%	7%

EXAMPLE 6

Disruption of Normal Rat Kidney (NRK) Cell Adhesion

NRK cells express E-cadherin, and monolayer cultures of these cells exhibit a cobblestone morphology. This Example illustrates the ability of a representative cyclic peptide to disrupt NRK cell adhesion.

NRK cells (ATCC #1571-CRL) were plated at 10 – 20,000 cells per 35mm tissue culture flasks containing DMEM with 10% FCS and sub-cultured periodically (Laird et al., *J. Cell Biol. 131*:1193-1203, 1995). Cells were harvested and replated in 35mm tissue culture flasks containing 1 mm coverslips and incubated until 50–65% confluent (24-36 hours). At this time, coverslips were transferred to a 24-well plate, washed once with fresh DMEM and exposed to cyclic peptide solutions (N-Ac-<u>CHAVC</u>-NH_2 (SEQ ID NO:8) and N-Ac-<u>CHGVC</u>-NH_2 (SEQ ID NO:9)) at a concentration of 1mg/mL for 24 hours. Fresh peptide solutions were then added and the cells were left for an additional 24 hours. Cells were fixed with 100% methanol for 10 minutes and then washed three times with PBS. Coverslips were blocked for 1 hour in 2% BSA/PBS and incubated for a further 1 hour in the presence of mouse anti-E-cadherin antibody (Transduction Labs, Lexington, KY; 1:250 dilution). Primary and secondary antibodies were diluted in 2% BSA/PBS. Following incubation in the primary antibody, coverslips were washed three times for 5 minutes each in PBS and incubated for 1 hour with donkey anti-mouse antibody conjugated to fluorescein (Jackson Immuno Research, West Grove, PA; diluted 1:200). Following a further wash in PBS (3 x 5 min) coverslips were mounted and viewed by confocal microscopy.

The peptide N-Ac-<u>CHAVC</u>-NH_2 (SEQ ID NO:8) disrupted NRK cell adhesion Figure 13D, compare to 13A), whereas N-Ac-<u>CHGVC</u>-NH_2 (SEQ ID NO:9) had no affect on cell adhesion (Figure 13C). In the presence of N-Ac-<u>CHGVC</u>-NH_2 (SEQ ID NO:9), the NRK cells formed tightly adherent monolayers with a cobblestone morphology. They also expressed E-cadherin, as judged by immunofluorescent staining protocols (Laird et al., *J. Cell Biol. 131*:1193-1203, 1995) (Figure 14C). In contrast, the NRK cells which were treated with N-Ac-<u>CHAVC</u>-NH_2 (SEQ ID NO:8) did not

adhere to one another and failed to form a contiguous monolayer (Figure 13D). Furthermore, these cells expressed greatly reduced levels of E-cadherin (Figure 14D). These data demonstrate that N-Ac-CHAVC-NH_2 (SEQ ID NO:8) is capable of disrupting NRK cell adhesion.

EXAMPLE 7

Enhancement of Human Skin Permeability

The epithelial cells of the skin (known as keratinocytes) express E-cadherin. This Example illustrates the use of a representative cyclic peptide to enhance the permeability of human skin.

Abdominal skin from humans at autopsy within 24 hours of death was used in these assays. The effect of N-Ac-CHAVC-NH_2 (SEQ ID NO:8) and N-Ac-CHGVC-NH_2 (SEQ ID NO:9), used at a concentration of 500 µg/ml or 2.5 mg/ml, on the penetration of two fluorescent markers, Oregon Green 488 (charge -1, MW 386 daltons) and Rhodamine Green 3000 Dextran (no charge, MW 3000 daltons) through human skin was then evaluated utilizing a Franz Cell apparatus (Franz, *Curr. Prob. Dermatol.* *7*:58-68, 1978; Franz, *J. Invest. Dermatol.* *64*:190-195, 1975). The peptides and markers were dissolved in sterile phosphate buffer, pH 7.2, and phosphate buffer was used as the receptor fluid. 150 µl of solution containing 0.2 mg Oregon Green and 1.0 mg Rhodamine Green was used to evaluate 500 µg/ml peptide; 200 µl of solution containing 0.05 mg Oregon Green and 1.250 mg Rhodamine Green was used to evaluate 2.5 mg/ml peptide. The solution was placed on top of the epidermal side of the skin, and the penetration of the markers through the skin was assessed using a fluorescent spectrophotometric method (in a Perkin Elmer 650-105 Fluorescence Spectrophotometer, and comparing the reading to a standard curve) at 6, 12, 24, 36, and 48 hours after the start of the experiment. The fluorescent units were converted to a concentration unit of microgram/5ml (volume of the receiver compartment) using a standard curve and regression analysis equations. The curve was linear for the concentrations tested for both markers (r^2 = 1 for OrG and 0.997 for RhG). For each peptide and marker combination, the experiment was performed in triplicate.

At 500 µg/ml, N-Ac-CHAVC-NH_2 (SEQ ID NO:8; sample #1) slightly increased the penetration of Oregon Green through the skin, as compared to the effect of N-Ac-CHGVC-NH_2 (SEQ ID NO:9; sample #3) on the penetration of this marker (Table 3 and Figure 18). The penetration of Rhodamine Green through the skin was

significantly increased in the presence of N-Ac-CHAVC-NH_2 (SEQ ID NO:8), in comparison to N-Ac-CHGVC-NH_2 (SEQ ID NO:9) (Table 4 and Figure 19).

At 2.5 mg/ml, N-Ac-CHAVC-NH_2 (SEQ ID NO:8; sample #1) increased the penetration of Oregon Green through the skin, as compared to the effect of N-Ac-CHGVC-NH_2 (SEQ ID NO:9; sample #3) on the penetration of this marker (Table 3 and Figure 20). The penetration of Rhodamine Green through the skin was significantly increased in the presence of N-Ac-CHAVC-NH_2 (SEQ ID NO:8), in comparison to N-Ac-CHGVC-NH_2 (SEQ ID NO:9) (Table 4 and Figure 21).

Table 3

*Percutaneous absorption concentration (mg/5ml) for Oregon Green™ 488 as a function of time

#Sample#	t = 6 hours	t = 12 hours	t= 24 hours	t = 36 hours	t = 48 hours
500 µg/ml Peptide					
1Sample#1	0.028	0.096	0.470	0.544	0.665
2Sample#1	0.167	0.322	1.096	1.56	1.725
3Sample#1	0.058	0.352	0.773	0.902	0.971
Mean Sample#1	0.084	0.225	0.780	1.00	1.120
1Sample#3	0.098	0.200	0.709	0.769	0.923
2Sample#3	0.022	0.107	0.864	0.923	1.021
3Sample#3	0.045	0.088	0.522	0.714	0.764
Mean Sample#3	0.055	0.132	0.698	0.802	0.902
2.5 mg/ml Peptide					
1Sample#1	0.14	0.44	0.67	0.76	0.83
2Sample#1	0.11	0.32	0.33	0.88	0.56
3Sample#1	0.16	0.45	0.63	0.99	1.06
Mean Sample #1	0.14	0.40	0.54	0.88	0.82
1Sample#3	0.04	0.11	0.12	0.23	0.36
2Sample#3	0.01	0.04	0.11	0.22	0.26
3Sample#3	0.06	0.08	0.26	0.29	0.46
Mean Sample #3	0.04	0.07	0.16	0.25	0.36
no dye	0	0	0	0	0
no dye	0	0	0	0	0

* Defined as amount found in the receiver compartment (volume = 5 ml)

Table 4

*Percutaneous absorption concentration (mg/5ml) for
Dextran Rhodamine Green 3000 as a function of time

#Sample#	t = 6 hours	t = 12 hours	t= 24 hours	t = 36 hours	t = 48 hours
500 µg/ml Peptide					
1Sample#1	0.4	3.0	16.174	21.044	25.747
2Sample#1	0.8	2.0	4.074	5.556	6.481
3Sample#1	1.2	5.556	13.158	17.565	27.826
Mean Sample#1	0.8	3.52	11.15	14.72	20.02
1Sample#3	0.2	0.6	1.0	1.0	1.8
2Sample#3	0.3	1.0	1.4	1.6	5.370
3Sample#3	0.2	0.4	0.8	1.0	1.8
Mean Sample#3	0.23	0.67	1.07	1.2	2.99
2.5 mg/ml Peptide					
1Sample#1	24.52	45.35	66.28	120.0	146.79
2Sample#1	2.4	25.22	35.22	42.36	47.00
3Sample#1	11.05	23.83	44.85	51.50	60.1
Mean Sample #1	12.66	31.47	48.78	71.28	133.56
1Sample#3	1.8	17.02	27.47	33.06	40.86
2Sample#3	0.2	2.0	5.56	5.79	8.25
3Sample#3	3.8	7.89	13.9	20.35	27.48
Mean Sample #3	1.93	8.97	15.64	19.73	25.53
no dye	0	0	0	0	0
no dye	0	0	0	0	0

* Defined as amount found in the receiver compartment (volume = 5 ml)

EXAMPLE 8

Disruption of Human Ovarian Cancer Cell Adhesion

This Example further illustrates the ability of representative cyclic peptides to disrupt human ovarian cancer cell adhesion.

The human ovarian cancer cell line OVCAR-3, which expresses E-cadherin, was used in these experiments. Cells were cultured in RPMI supplemented with insulin and containing 20% FCS. Cells were grown in T-250 culture flasks and maintained by periodic subculturing. Cells were harvested from flasks and seeded in individual wells of 96-well culture dishes (surface area of each well was $0.32cm^2$) at a density of 50,000 cells per well in 0.1 ml media containing the cyclic peptides (at concentrations of 1, 0.1, or 0.01mg/ml). Media control wells were also established. Cultures were evaluated periodically by microscopic examination under both bright field and phase contrast conditions, and were maintained for 48 hours. N-Ac-CHAVC-NH_2 (SEQ ID NO:8) was found to be inactive within this assay at these concentrations. However, the cyclic peptide N-Ac-CHAVSC-NH_2 (SEQ ID NO:14) disrupted OVCAR-3 adhesion (Figures 15A-C)). This data demonstrates that N-Ac-CHAVSC-NH_2 (SEQ ID NO:14) specifically affects cells that express E-cadherin.

EXAMPLE 9

Disruption of Melanoma Cell Adhesion

This Example illustrates the ability of a representative cyclic peptide to disrupt melanoma cell adhesion.

Melanoma ME115 cells (kindly provided by Meenhard Herlyn, Wistar Institute, Philadelphia, PA) were plated on glass coverslips and cultured for 24 hours in 50% keratinocyte growth medium (Clonetics, San Diego, CA) and 50% L15. Fresh medium containing the cyclic peptides (final concentration 500 µg/mL media) N-Ac-CHAVC-NH_2 (SEQ ID NO:8) or N-Ac-CHGVC-NH_2 (SEQ ID NO:9) was then added. Following 24 hours of culture in the presence of the peptides, the medium was removed

and fresh medium containing the peptides was added. The cells were fixed 24 hours later with cold methanol and stored in phosphate buffered saline (PBS).

Coverslips were blocked for 1 hour in 3% ovalbumin/PBS and incubated for a further 1 hour in the presence of rabbit pan-cadherin antibody (Sigma Chemical Co., St. Louis, MO) diluted 1:500. Primary and secondary antibodies were diluted in PBS containing 6% normal goat serum. Following incubation in the primary antibody, coverslips were washed 3 times for 5 minutes each in PBS and incubated for 1 hour in goat anti-rabbit immunoglobulin G conjugated to fluorescein (Kiekegard and Perry, South San Francisco, CA) diluted 1:100. Following a further wash in PBS (3 x 5 minutes) coverslips were mounted in Vectashield (Vector Labs, Burlingame, CA) and viewed with a Zeiss infinity corrected microscope.

Photographs, shown in Figure 16, show an absence of cell membrane staining and the appearance of bright intracellular vesicular staining in cells treated with N-Ac-<u>CHAVC</u>-NH_2 (SEQ ID NO:8). In contrast, cells exposed to N-Ac-<u>CHGVC</u>-NH_2 (SEQ ID NO:9) displayed cadherin staining all over the cell membrane. Occasionally, the staining concentrated at points of cell-cell contact. These results indicate that the representative cyclic peptide N-Ac-<u>CHAVC</u>-NH_2 (SEQ ID NO:8) disrupts melanoma cell adhesion.

EXAMPLE 10

Disruption of Breast Cancer Cell Adhesion

This Example illustrates the ability of a representative cyclic peptide to disrupt human breast epithelial cell adhesion.

A1N4 human breast epithelial cells (kindly provided by Martha Stampfer, Lawrence Berkeley Laboratory, Berkeley, CA) were plated on glass coverslips and cultured in F12/DME containing 0.5% FCS and 10 ng/mL EGF for 24 hours. Fresh medium containing the cyclic peptides (final concentration 500µg/mL media) N-Ac-<u>CHAVC</u>-NH_2 (SEQ ID NO:8) or N-Ac-<u>CHGVC</u>-NH_2 (SEQ ID NO:9) was then added. Following 24 hours of culture in the presence of the peptides, the

medium was removed and fresh medium containing the peptides was added. The cells were fixed 24 hours later with cold methanol and stored in phosphate buffered saline (PBS).

Coverslips were blocked for 1 hour in 3% ovalbumin/PBS and incubated for a further 1 hour in the presence of 1 µg/mL mouse anti-E-cadherin antibody (Zymed, Gaithersburg, MD). Primary and secondary antibodies were diluted in PBS containing 6% normal goat serum. Following incubation in the primary antibody, coverslips were washed 3 times for 5 minutes each in PBS and incubated for 1 hour with goat anti-mouse conjugated to fluorescein (Kiekegard and Perry, South San Francisco, CA) diluted 1:100. Following a further wash in PBS (3 x 5 minutes) coverslips were mounted in Vectashield (Vector Labs, Burlingame, CA) and viewed with a Zeiss infinity corrected microscope.

Photographs, shown in Figures 17 A and B, show reduced E-cadherin staining with a stitched appearance in cells treated with N-Ac-<u>CHAVC</u>-NH_2 (SEQ ID NO:8). In addition, holes are present in the monolayer where the cells have retracted from one another. In contrast, cells exposed to N-Ac-<u>CHGVC</u>-NH_2 (SEQ ID NO:9) displayed E-cadherin staining concentrated at points of cell-cell contact and formed a tightly adherent monolayer.

EXAMPLE 11

Toxicity and Cell Proliferation Studies

This Example illustrates the initial work to evaluate the cytotoxic effects of representative cyclic peptides.

N-Ac-<u>CHAVC</u>-NH_2 (SEQ ID NO:8) and the control peptide N-Ac-<u>CHGVC</u>-NH_2 (SEQ ID NO:9) were evaluated for possible cytotoxic effects on human microvascular endothelial (HMVEC; Clonetics), human umbilical vein endothelial (HUVEC; ATCC #CRL-1730), IAFp2 (human fibroblast cell line; Institute Armand-Frapier, Montreal, Quebec), WI-38 (human fibroblast cell line; ATCC #CCL-75), MDA-MB231 (human breast cancer cell line; ATCC #HTB-26), and PC-3 (human

prostate cancer cell line; ATCC #CRL-1435) cells utilizing the MTT assay (Plumb et al., *Cancer Res.* *49*:4435-4440; 1989). Neither of the peptides was cytotoxic at concentrations up to and including 100 μM. Similarly, neither of the peptides was capable of inhibiting the proliferation of the above cell lines at concentrations up to 100 μM, as judged by ^{3}H-thymidine incorporation assays.

In fact, none of the compounds tested thus far show any cytotoxicity at concentrations up to and including 100 μM (Table 5 and 6). However, N-Ac-<u>CHAVSC</u>-NH_2 (SEQ ID NO:14), N-Ac-<u>CHGVSC</u>-NH_2, N-Ac-<u>CVAHC</u>-NH_2, N-Ac-<u>CVGHC</u>-NH_2 and N-Ac-<u>CSHAVSSC</u>-NH_2 (SEQ ID NO:18) inhibited the proliferation of HUVEC at concentrations (IC_{50} values) of 57 μM, 42 μM, 8 μM, 30 μM and 69 μM respectively, as judged by ^{3}H-thymidine incorporation assays. N-Ac-<u>CSHAVSSC</u>-NH_2 (SEQ ID NO:18) also inhibited the proliferation of MDA-MB231 cells at a concentration of 76 μM and HMVEC cells at a concentration of 70 μM (Tables 5 and 6). N-Ac-<u>CHAVSC</u>-NH_2 (SEQ ID NO:14) inhibited the proliferation of MDA-MB231 cells at a concentration of 52 μM.

Table 5

Evaluation of Peptides for Cytotoxicity and Capacity to Inhibit Cell Proliferation of Normal Cells (IC_50 in µM)

Peptide	Normal Cells							
	HMVEC		HUVEC		IAFp2		WI-38	
	Cell prol	Cytotox	Cell Prol	Cytotox	Cell Prol	Cytotox	Cell Prol	Cytotox
N-Ac-CHGVC-NH_2 (control for #1)	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM
N-Ac-CHAVC-NH_2 (#1)	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM
H-CHGVC-NH_2 (control for #2)	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM
H-CHAVC-NH_2 (#2)	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM
N-Ac-CHGVSC-NH_2 (control for #18)	>100µM	>100µM	42µM	>100µM	>100µM	>100µM	>100µM	>100µM
N-Ac-CHAVSC-NH_2 (#18)	>100µM	>100µM	57µM	>100µM	>100µM	>100µM	>100µM	>100µM
N-Ac-CSHGVC-NH_2 (control for #16)	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM
N-Ac-CSHAVC-NH_2 (#16)	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM
N-Ac-CAHGVDC-NH_2 (control for #22)	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM
N-Ac-CAHAVDC-NH_2 (#22)	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM
N-Ac-KHGVD-NH_2 (control for #26)	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM

N-Ac-KHAVD-NH_2 (#26)	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM
H-CAHGVDC-NH_2 (control for #45)	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM
H-CAHAVDC-NH_2 (#45)	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM
H-CAHGVDIC-NH_2 (control for #47)	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM
H-CAHAVDIC-NH_2 (#47)	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM
N-Ac-CVGHC-NH_2 (control for #32)	>100µM	>100µM	30µM	>100µM	>100µM	>100µM	>100µM	>100µM
N-Ac-CVAHC-NH_2 (#32)	>100µM	>100µM	8µM	>100µM	>100µM	>100µM	>100µM	>100µM
N-Ac-CAHGVDIC-NH_2 (control for #14)	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM
N-Ac-CAHAVDIC-NH_2 (#14)	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM
N-Ac-CSHGVSSC-NH_2 (control for #24)	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM	>100µM
N-Ac-CSHAVSSC-NH_2• (#24)	70µM	>100µM	69µM	>100µM	>100µM	>100µM	>100µM	>100µM

• Incompletely soluble in RPMI at 1 mM

Table 6

Evaluation of Peptides for Cytotoxicity and Capacity to Inhibit Cell Proliferation of Tumoral Cells (IC_50 in μM)

Peptide	Tumoral Cells			
	MDA-MB231		PC-3	
	Cell Prol	Cytotox	Cell Prol	Cytotox
N-Ac-CHGVC-NH_2 (control for #1)	>100μM	>100μM	>100μM	>100μM
N-Ac-CHAVC-NH_2 (#1)	>100μM	>100μM	>100μM	>100μM
H-CHGVC-NH_2 (control for #2)	>100μM	>100μM	>100μM	>100μM
H-CHAVC-NH_2 (#2)	>100μM	>100μM	>100μM	>100μM
N-Ac-CHGVSC-NH_2 (control for #18)	>100μM	>100μM	>100μM	>100μM
N-Ac-CHAVSC-NH_2*(#18)	52μM	>100μM	>100μM	>100μM
N-Ac-CSHGVC-NH_2 (control for #16)	>100μM	>100μM	>100μM	>100μM
N-Ac-CSHAVC-NH_2 (#16)	>100μM	>100μM	>100μM	>100μM
N-Ac-CAHGVDC-NH_2 (control for #22)	>100μM	>100μM	>100μM	>100μM
N-Ac-CAHAVDC-NH_2 (#22)	>100μM	>100μM	>100μM	>100μM
N-Ac-KHGVD-NH_2 (control for #26)	>100μM	>100μM	>100μM	>100μM
N-Ac-KHAVD-NH_2 (#26)	>100μM	>100μM	>100μM	>100μM
H-CAHGVDC-NH_2 (control for #45)	>100μM	>100μM	>100μM	>100μM

H-CAHAVDC-NH_2 (#45)	>100μM	>100μM	>100μM	>100μM
H-CAHGVDIC-NH_2 (control for #47)	>100μM	>100μM	>100μM	>100μM
H-CAHAVDIC-NH_2 (#47)	>100μM	>100μM	>100μM	>100μM
N-Ac-CVGHC-NH_2 (control for #32)	>100μM	>100μM	>100μM	>100μM
N-Ac-CVAHC-NH_2 (#32)	>100μM	>100μM	>100μM	>100μM
N-Ac-CAHGVDIC-NH_2 (control for #14)	>100μM	>100μM	>100μM	>100μM
N-Ac-CAHAVDIC-NH_2 (#14)	>100μM	>100μM	>100μM	>100μM
N-Ac-CSHGVSSC-NH_2 (control for #24)	>100μM	>100μM	>100μM	>100μM
N-Ac-CSHAVSSC-NH_2 * (#24)	76μM	>100μM	>100μM	>100μM

* Incompletely soluble in RPMI at 1 mM

Example 12

Chronic Toxicity Study

This Example illustrates a toxicity study performed using a representative cyclic peptide.

Varying amounts of H-CHAVC-NH_2 (SEQ ID NO:8; 2 mg/kg, 20 mg/kg and 125 mg/kg) were injected into mice intraperitoneally every day for three days. During the recovery period (days 4-8), animals were observed for clinical symptoms. Body weight was measured (Table 22) and no significant differences occurred. In addition, no clinical symptoms were observed on the treatment or recovery days. Following the four day recovery period, autopsies were performed and no abnormalities were observed.

Example 13

Stability of Cyclic Peptide in Blood

This Example illustrates the stability of a representative cyclic peptide in mouse whole blood.

50 μl of a stock solution containing 12.5 μg/ml H-CHAVC-NH_2 (SEQ ID NO:8) was added to mouse whole blood and incubated at 37°C. Aliquots were removed at intervals up to 240 minutes, precipitated with acetonitrile, centrifuged and analyzed by HPLC. The results (Table 7 and Figure 23) are expressed as % remaining at the various time points, and show generally good stability in blood.

Table 7

Stability of Representative Cyclic Peptide in Mouse Whole Blood

Time (Min.)	Area 1	Area 2	Average	% Remaining
0	341344	246905	294124.5	100.00
10	308924	273072	290998	98.94
20	289861	220056	254958.5	86.68
30	353019	310559	331789	112.81
45	376231	270860	323545.5	110.00
60	373695	188255	280975	95.53
90	435555	216709	326132	110.88
120	231694	168880	200287	68.10
240	221952	242148	232050	78.90

From the foregoing, it will be evident that although specific embodiments of the invention have been described herein for the purpose of illustrating the invention, various modifications may be made without deviating from the spirit and scope of the invention.

Claims

What is claimed is:

1. A method for inhibiting synaptic stability in a mammal, comprising administering to a mammal a therapeutically effective amount of a cell adhesion modulating agent that inhibits cadherin-mediated cell adhesion, wherein the agent comprises a cyclic peptide having a peptide ring, and wherein the sequence His-Ala-Val is present within the peptide ring, and thereby inhibiting synaptic stability in the mammal.

2. A method according to claim 1, wherein the cyclic peptide has the formula:

$$(Z_1)\text{-}(Y_1)\text{-}(X_1)\text{-His-Ala-Val-}(X_2)\text{-}(Y_2)\text{-}(Z_2)$$

wherein X_1; and X_2 are optional, and if present, are independently selected from the group consisting of amino acid residues and combinations thereof in which the residues are linked by peptide bonds, and wherein X_1 and X_2 independently range in size from 0 to 10 residues, such that the sum of residues contained within X_1 and X_2 ranges from 1 to 12;

wherein Y_1 and Y_2 are independently selected from the group consisting of amino acid residues, and wherein a covalent bond is formed between residues Y_1 and Y_2; and

wherein Z_1 and Z_2 are optional, and if present, are independently selected from the group consisting of amino acid residues and combinations thereof in which the residues are linked by peptide bonds.

3. A method according to claim 2, wherein Z_1 is not present and Y_1 comprises an N-acetyl group.

4. A method according to claim 2, wherein Z_2 is not present and Y_2 comprises a C-terminal amide group.

5. A method according to claim 2, wherein Y_1 and Y_2 are covalently linked via a disulfide bond.

6. A method according to claim 5, wherein Y_1 and Y_2 are each independently selected from the group consisting of penicillamine, β,β-tetramethylene cysteine, β,β-pentamethylene cysteine, β-mercaptopropionic acid, β,β-pentamethylene-β-mercaptopropionic acid, 2-mercaptobenzene, 2-mercaptoaniline, 2-mercaptoproline and derivatives thereof.

7. A method according to claim 5, wherein Y_1 and Y_2 are cysteine residues or derivatives thereof.

8. A method according to claim 7, wherein the cyclic peptide comprises the sequence CHAVC (SEQ ID NO:8).

9. A method according to claim 8, further comprising an N-acetyl group.

10. A method according to claim 8, further comprising a C-terminal amide group.

11. A method according to claim 7, wherein the cyclic peptide comprises a sequence selected from the group consisting of CHAVDC (SEQ ID NO:48), CAHAVC (SEQ ID NO:49), CAHAVDC (SEQ ID NO:16), CAHAVDIC (SEQ ID NO:10), CRAHAVDC (SEQ ID NO:50), CLRAHAVC (SEQ ID NO:51), CLRAHAVDC (SEQ ID NO:52), CSHAVC (SEQ ID NO:12), CHAVSC (SEQ ID NO:14), CSHAVSC (SEQ ID NO:53),

CSHAVSSC (SEQ ID NO:18), CHAVSSC (SEQ ID NO:54) and derivatives of the foregoing sequences having one or more C-terminal, N-terminal and/or side chain modifications.

12. A method according to claim 2, wherein Y_1 and Y_2 are covalently linked via an amide bond.

13. A method according to claim 12, wherein the amide bond is formed is formed between terminal functional groups.

14. A method according to claim 12, wherein the amide bond is formed between residue side-chains.

15. A method according to claim 12, wherein the amide bond is formed between one terminal functional group and one residue side chain.

16. A method according to claim 12, wherein:

(a) Y_1 is selected from the group consisting of lysine, ornithine, and derivatives thereof and Y_2 is selected from the group consisting of aspartate, glutamate and derivatives thereof; or

(b) Y_2 is selected from the group consisting of lysine, ornithine and derivatives thereof and Y_1 is selected from the group consisting of aspartate, glutamate and derivatives thereof.

17. A method according to claim 12, wherein the cyclic peptide comprises a sequence selected from the group consisting of KHAVD (SEQ ID NO:20), DHAVK (SEQ ID NO:55), KHAVE (SEQ ID NO:56), AHAVDI (SEQ ID NO:44), SHAVDSS (SEQ ID NO:57), KSHAVSSD (SEQ ID NO:45) and derivatives of the foregoing sequences having one or more C-terminal, N-terminal and/or side chain modifications.

18. A method according to claim 2, wherein Y_1 and Y_2 are covalently linked via a thioether bond.

19. A method according to claim 2, wherein Y_1 and Y_2 are each tryptophan or a derivative thereof, such that the covalent bond generates a $\delta_1\delta_1$-ditryptophan, or a derivative thereof.

20. A method according to claim 1, wherein the agent is linked to a targeting agent.

21. A method according to claim 1, wherein the agent is linked to a drug.

22. A method according to claim 1, wherein the agent is linked to a solid support.

23. A method according to claim 22, wherein the solid support is a polymeric matrix.

24. A method according to claim 23, wherein the solid support is selected from the group consisting of plastic dishes, plastic tubes, sutures, membranes, ultra thin films, bioreactors and microparticles.

25. A method according to claim 1, wherein the agent further comprises one or more of:

(a) a cell adhesion recognition sequence that is bound by an adhesion molecule other than a cadherin, wherein said cell adhesion recognition sequence is separated from any HAV sequence(s) by a linker; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than a cadherin.

26. A method according to claim 25, wherein the adhesion molecule is selected from the group consisting of integrins, occludin, N-CAM, desmogleins, desmocollins, fibronectin, laminin and other extracellular matrix proteins.

27. A method according to claim 1, wherein the agent is linked to a detectable marker.

28. A method according to claim 1, wherein the agent is present within a pharmaceutical composition that further comprises a pharmaceutically acceptable carrier.

29. A method according to claim 28, wherein the composition further comprises a drug.

30. A method according to claim 28, wherein the agent is present within a sustained-release formulation.

31. A method according to claim 28, wherein the pharmaceutical composition further comprises one or more of:

(a) a peptide comprising a cell adhesion recognition sequence that is bound by an adhesion molecule other than a cadherin; and/or

(b) an antibody or antigen-binding fragment thereof that specifically binds to a cell adhesion recognition sequence bound by an adhesion molecule other than a cadherin.

32. A method according to claim 31, wherein the adhesion molecule is selected from the group consisting of integrins, occludin, N-CAM, desmogleins, desmocollins, fibronectin, laminin and other extracellular matrix proteins.

33. A method according to claim 31, wherein the cell adhesion recognition sequence is KYSFNYDGSE (SEQ ID NO:62).

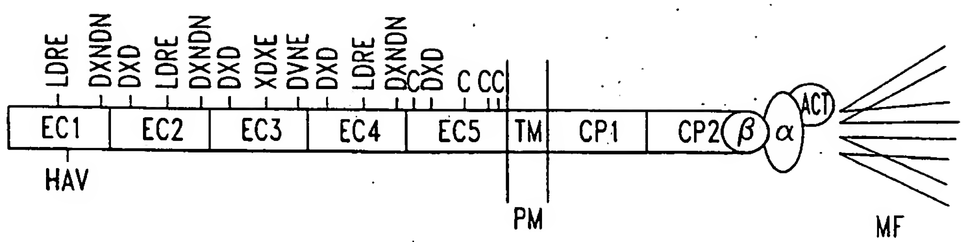
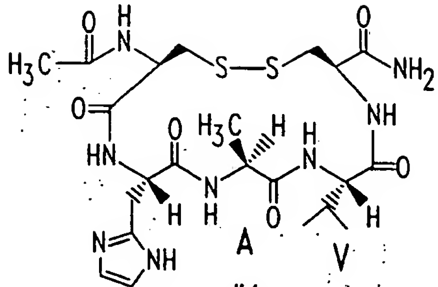

LDRE
DXNDN
DXD
LDRE
DXNDN
DXD
XDXE
DVNE
DXD
LDRE
DXNDN
C
DXD
C
CC
EC1
EC2
EC3
EC4
EC5
TM
CP1
CP2
β
α
ACT
HAV
PM
MF

Fig. 1

human N-cad	DWVIPPINLPENSRGPFPQELVRIRSDRDKNLSLRYSVTGPGADQPPTGIFILNPISGQLSVTKPLDREQ
mouse N-cad	DWVIPPINLPENSRGPFPQELVRIRSDRDKNLSLRYSVTGPGADQPPTGIFIINPISGQLSVTKPLDREL
cow N-cad	DWVIPPINLPENSRGPFPQELVRIRSDRDKNLSLRYSVTGPGADQPPTGIFIINPISGQLSVTKPLDREL
human P-cad	DWVVAPISVPENGKGPFPQRLNQLKSNKDRDTKIFYSITGPGADSPPEGVFAVEKETGWLLLNKPLDREE
mouse P-cad	EWVMPPIFVPENGKGPFPQRLNQLKSNKDRGTKIFYSITGPGADSPPEGVFTIEKESGWLLLHMPLDREK
human E-cad	DWVIPPISCPENEKGPFPKNLVQIKSNKDKEGKVFYSITGQGADTPPVGVFIIERETGWLKVTEPLDRER
mouse E-cad	DWVIPPISCPENEKGEFPKNLVQIKSNRDKETKVFYSITGQGADKPPVGVFIIERETGWLKVTQPLDREA

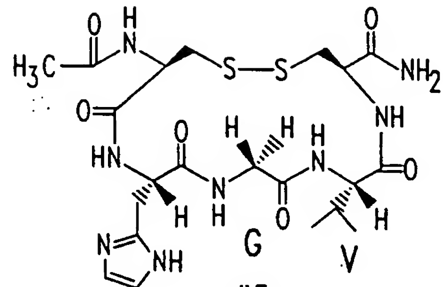
human N-cad	IARFHLRA**HAV**DINGNQVENPIDIVINVIDMNDNRPEF
mouse N-cad	IARFHLRA**HAV**DINGNQVENPIDIVINVIDMNDNRPEF
cow N-cad	IARFHLRA**HAV**DINGNQVENPIDIVINVIDMNDNRPEF
human P-cad	IAKYELFG**HAV**SENGASVEDPMNISIIVTDQNDHKPKF
mouse P-cad	IVKYELYG**HAV**SENGASVEEPMNISIIVTDQNDNKPKF
human E-cad	IATYTLFS**HAV**SSNGNAVEDPMEILITVTDQNDNKPEF
mouse E-cad	IAKYILYS**HAV**SSNGEAVEDPMEIVITVTDQNDNRPEF

Fig. 2



H #1

N-Ac-CHAVC-NH$_2$



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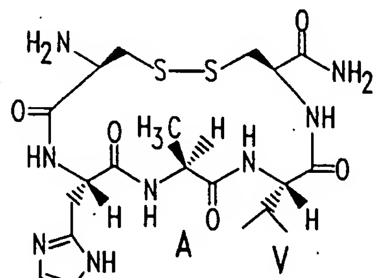
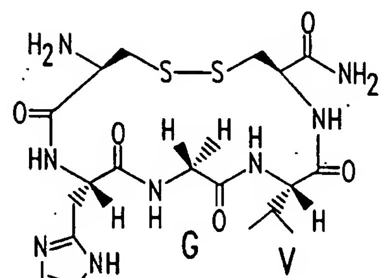
N-Ac-CHGVC-NH$_2$



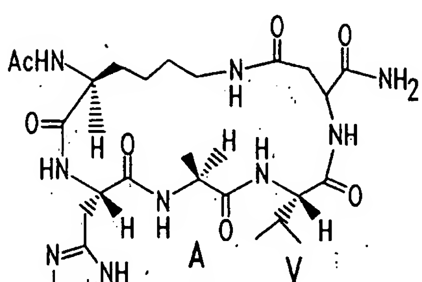
H #2

H-CHAVC-NH$_2$



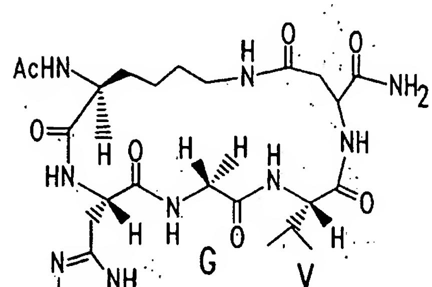
H #4

H-CHGVC-NH$_2$



H #26

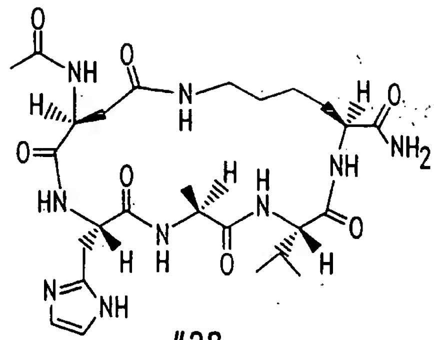
N-Ac-KHAVD-NH$_2$



H #27

N-Ac-KHGVD-NH$_2$

Fig. 3A



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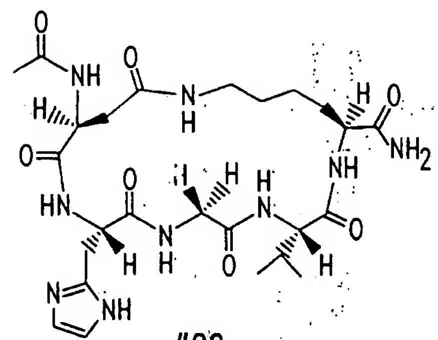
N-Ac-DHAVK-NH2



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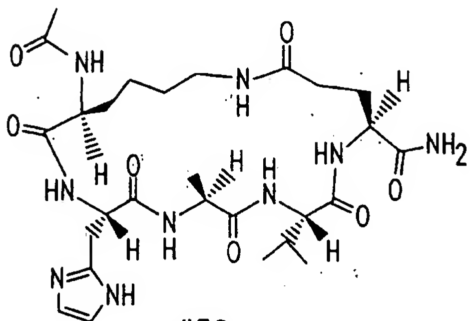
N-Ac-DHGVK-NH2



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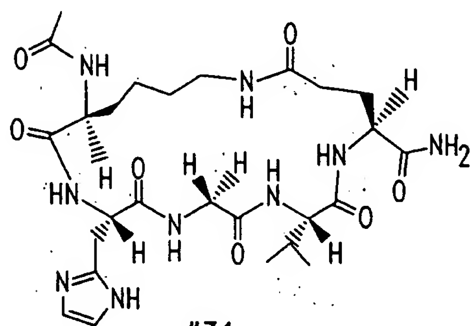
N-Ac-KHAVE-NH2



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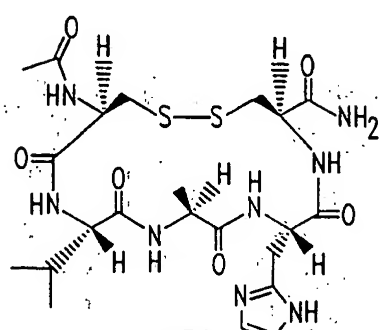
N-Ac-KHGVE-NH2



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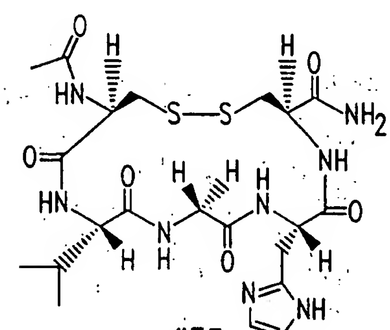
N-Ac-CVAHC-NH2



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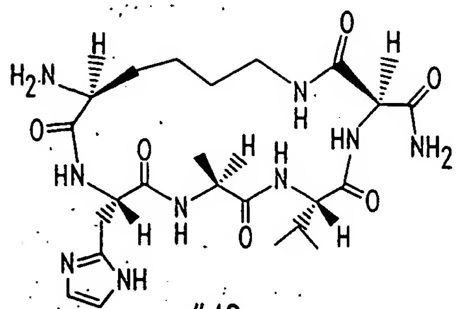
N-Ac-CVGHC-NH2

Fig. 3B



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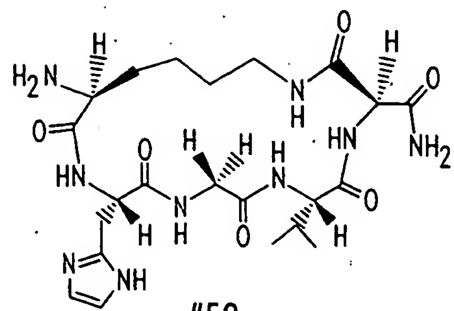
H-KHAVD-NH$_2$



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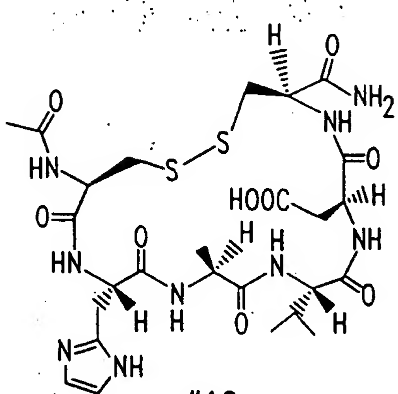
H-KHGVD-NH$_2$



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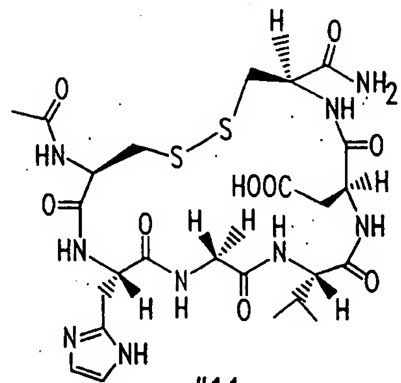
N-Ac-CHAVDC-NH$_2$



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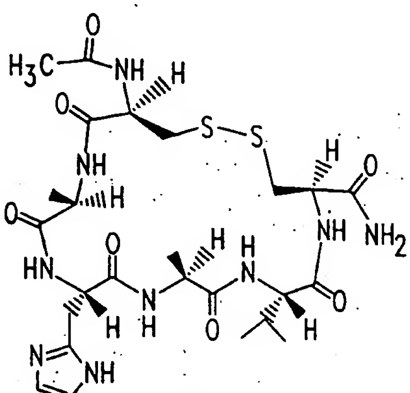

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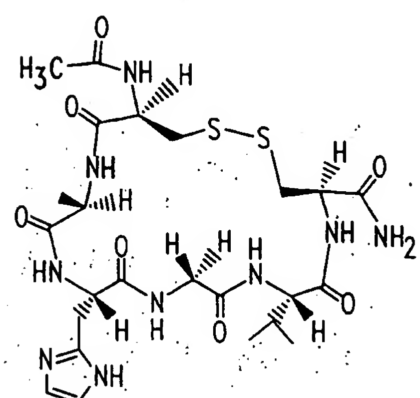
N-Ac-CAHAVC-NH$_2$



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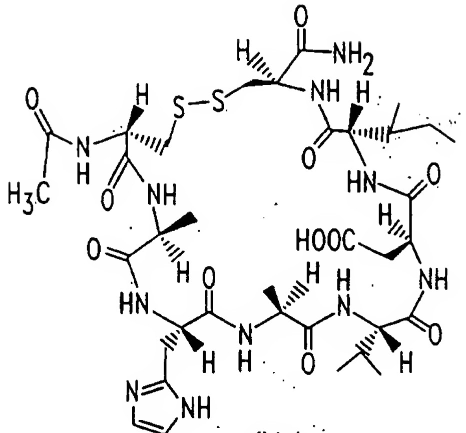
N-Ac-CAHGVC-NH$_2$

Fig. 3C



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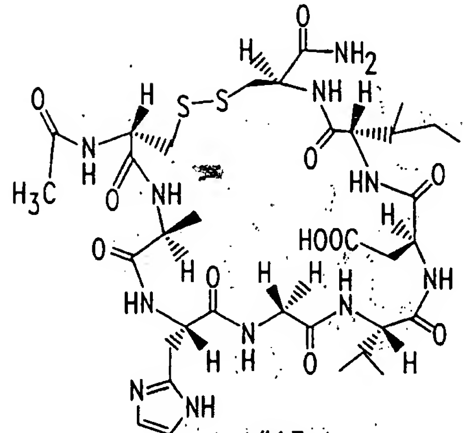
N-Ac-CAHAVDIC-NH_2



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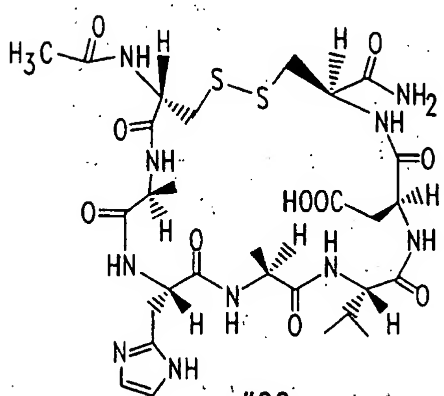
N-Ac-CAHGVDIC-NH_2



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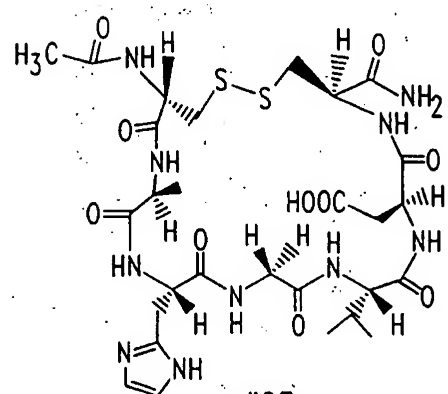
N-Ac-CAHAVDC-NH_2



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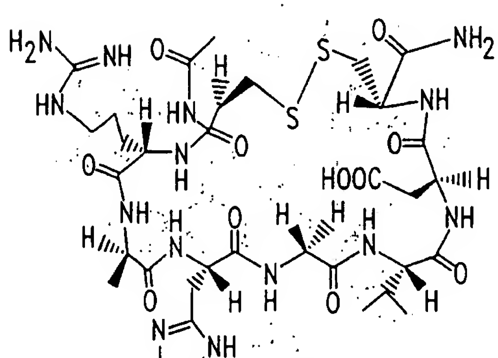
N-Ac-CAHGVDC-NH_2



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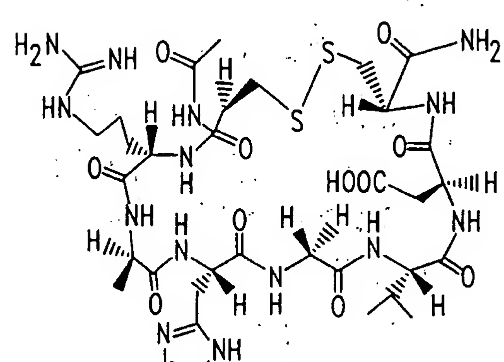
N-Ac-CRAHAVDC-NH_2



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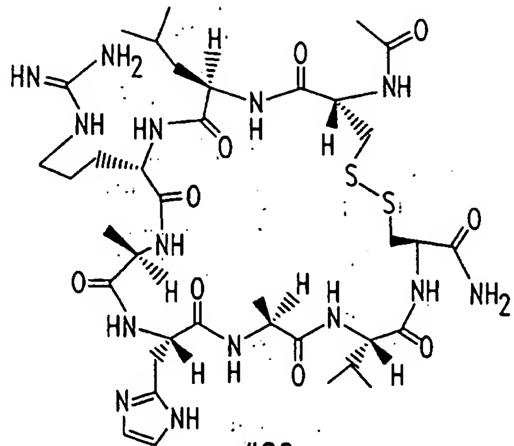
N-Ac-CRAHGVDC-NH_2

Fig. 3D



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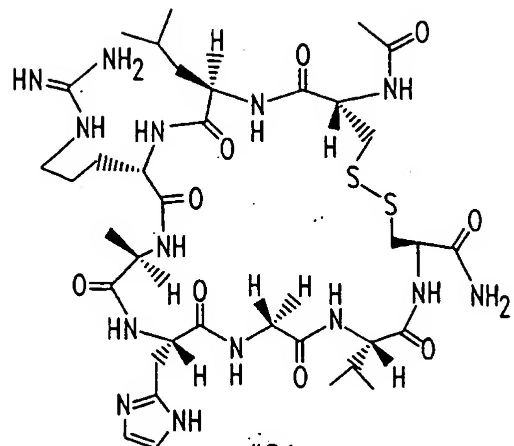
N-Ac-CLRAHAVC-NH$_2$



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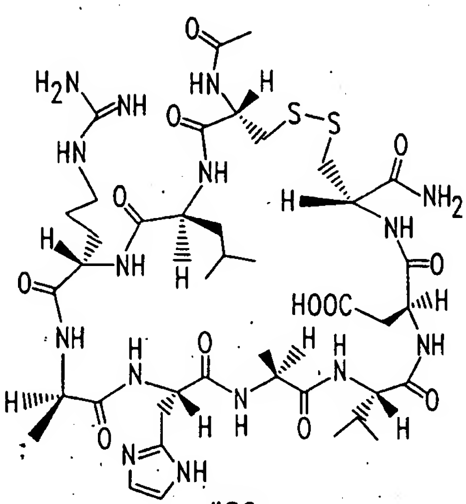
N-Ac-CLRAHGVC-NH$_2$



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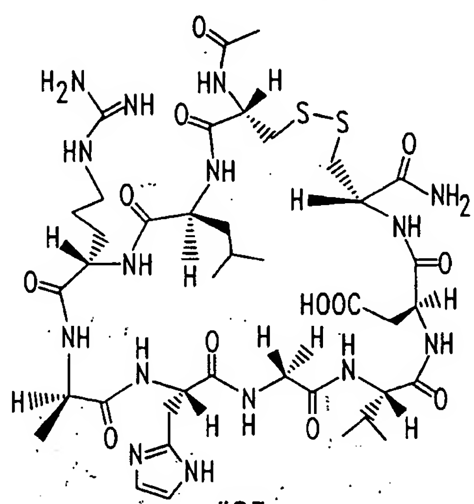
N-Ac-CLRAHAVDC-NH$_2$



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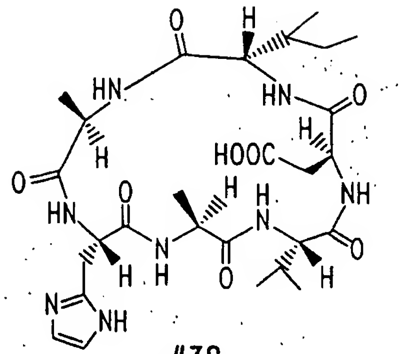
N-Ac-CLRAHGVDC-NH$_2$

Fig. 3E



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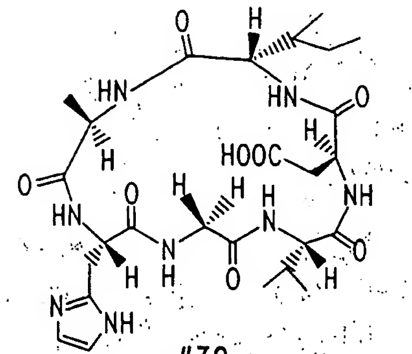
AHAVDI



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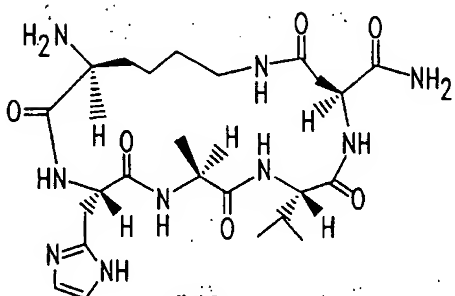
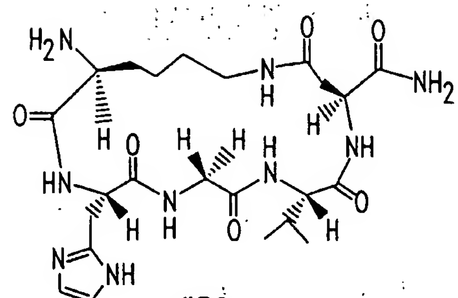
AHGVDI



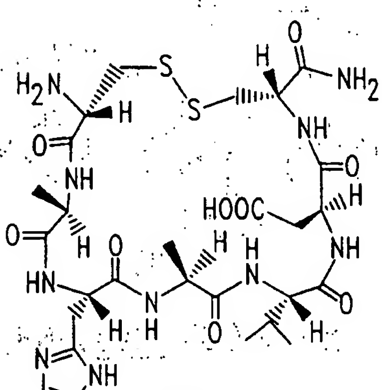
#49

H-KHAVD-NH_2



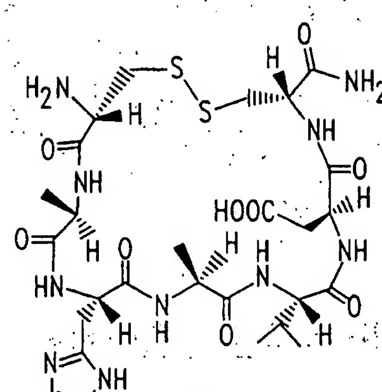
#50

H-KHGVD-NH_2



#45

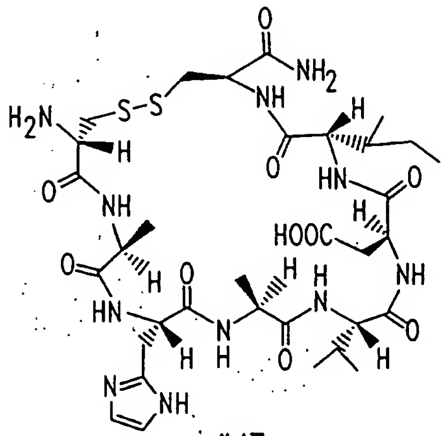
H-CAHAVDC-NH_2



#46

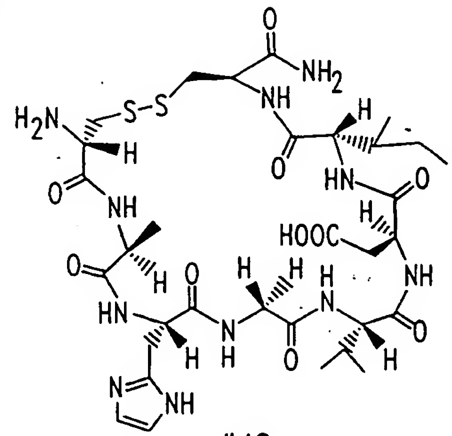
H-CAHGVDC-NH_2

Fig. 3F



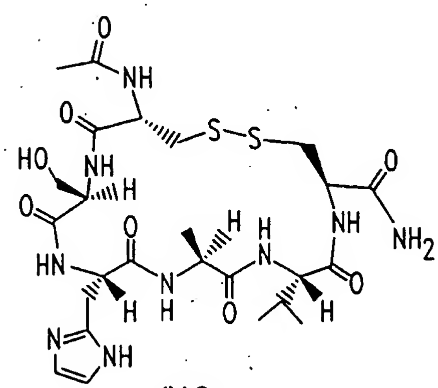
#47

H-<u>CAHAVDIC</u>-NH_2



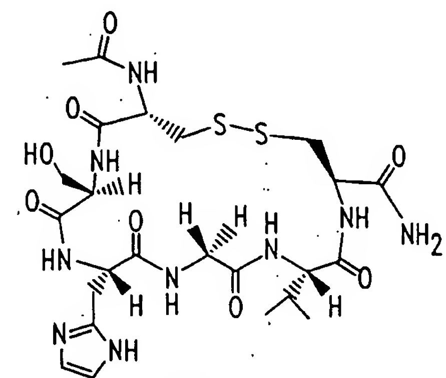
#48

H-<u>CAHGVDIC</u>-NH_2



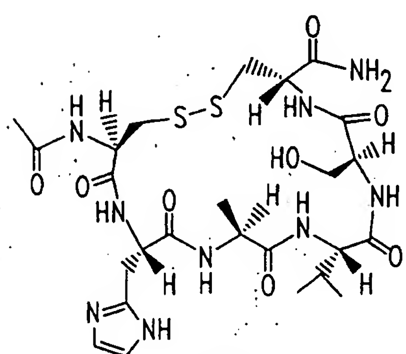
#16

N-Ac-<u>CSHAVC</u>-NH_2



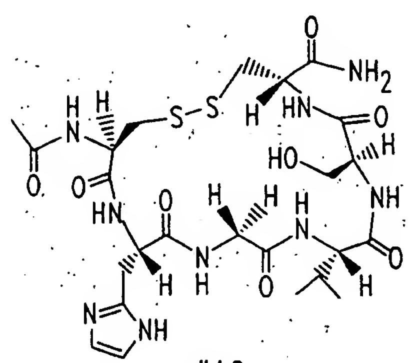
#17

N-Ac-<u>CSHGVC</u>-NH_2



#18

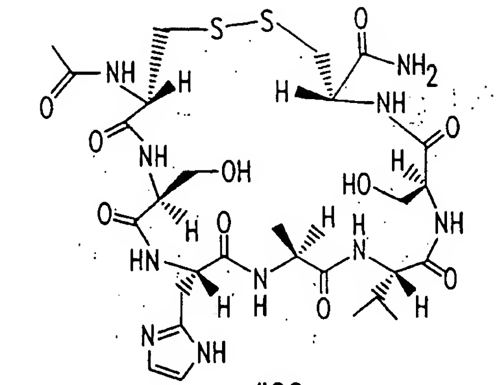
N-Ac-<u>CHAVSC</u>-NH_2



#19

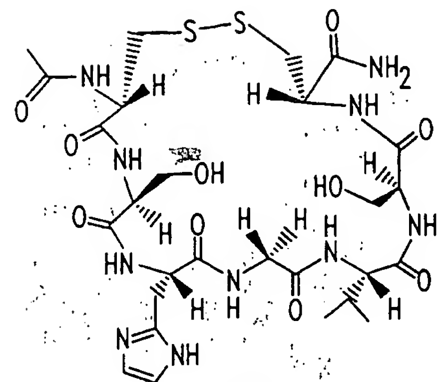
N-Ac-<u>CHGVSC</u>-NH_2

Fig. 3G



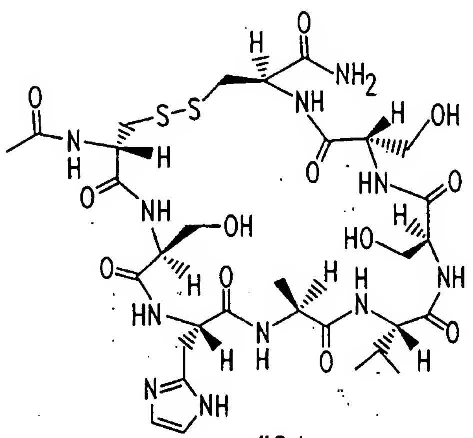
#20

N-Ac-<u>CSHAVSC</u>-NH_2



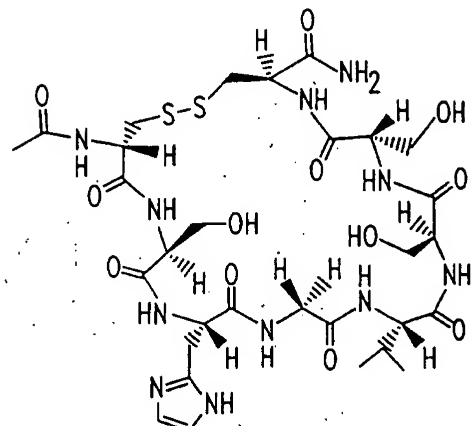
#21

N-Ac-<u>CSHGVSC</u>-NH_2



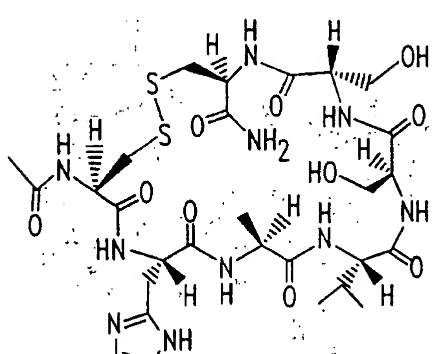
#24

N-Ac-<u>CSHAVSSC</u>-NH_2



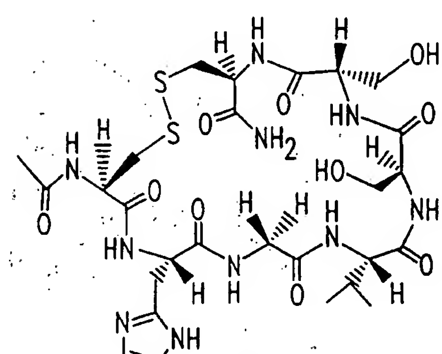
#25

N-Ac-<u>CSHGVSSC</u>-NH_2



#66

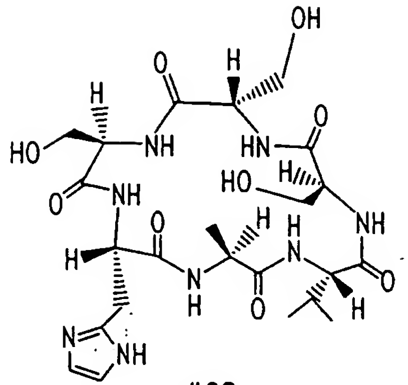
N-Ac-<u>CHAVSSC</u>-NH_2



#67

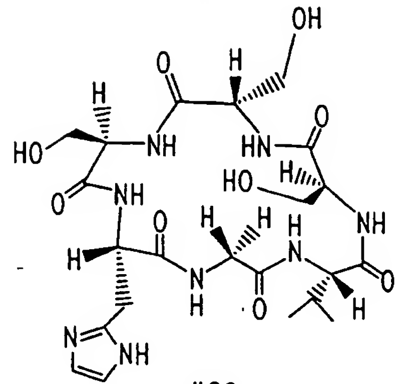
N-Ac-<u>CHGVSSC</u>-NH_2

Fig. 3H



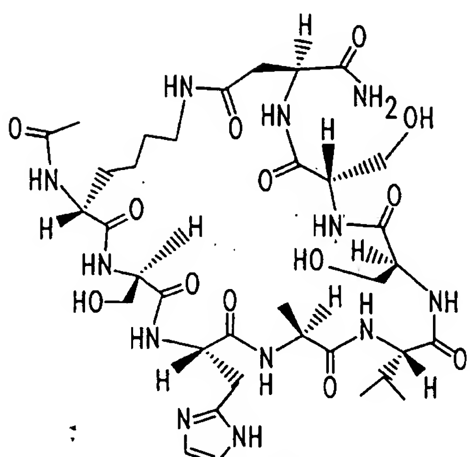
#68

SHAVSS



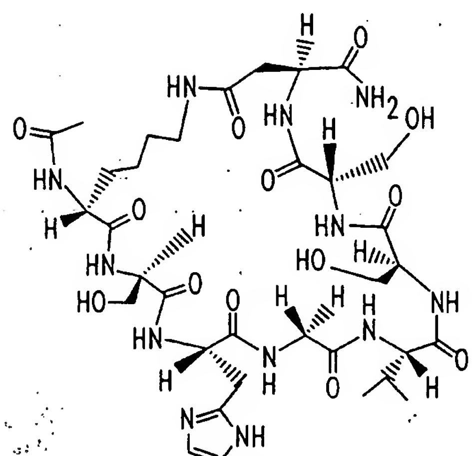
#69

SHGVSS



#70

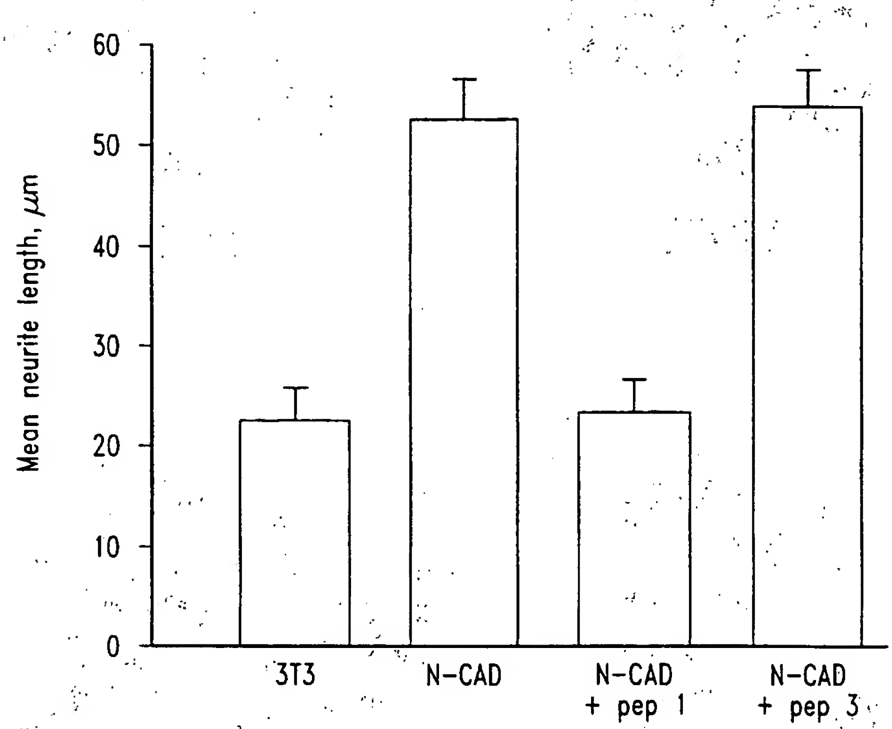
N-Ac-KSHAVSSD-NH_2



#71

N-Ac-KSHGVSSD-NH_2

Fig. 3I



Mean neurite length, μm
60
50
40
30
20
10
0
3T3
N-CAD
N-CAD
+ pep 1
N-CAD
+ pep 3

Fig. 4

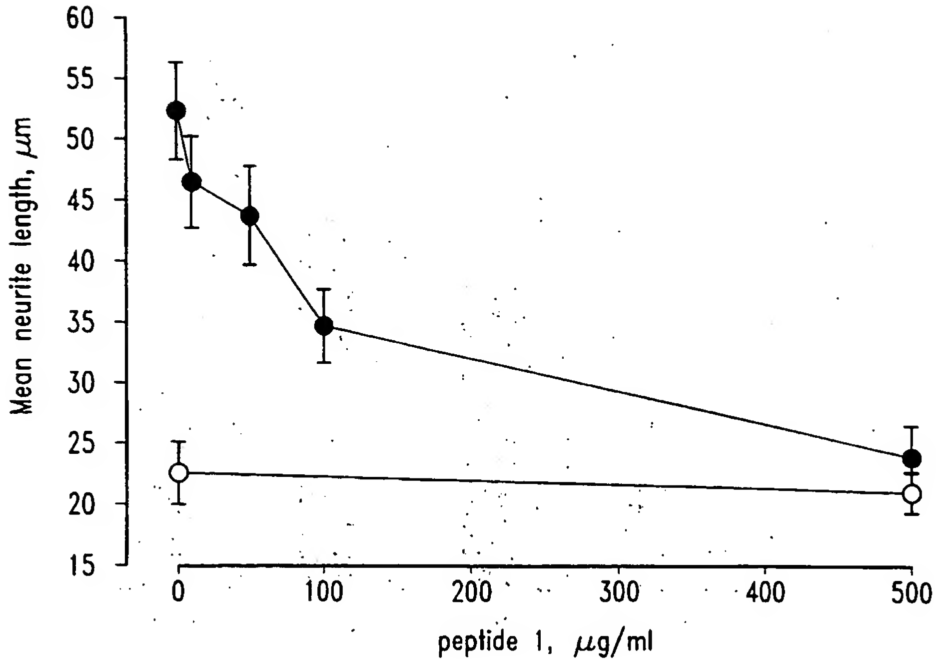

60
55
50
45
40
35
30
25
20
15
Mean neurite length, µm
0
100
200
300
400
500
peptide 1, µg/ml

Fig. 5

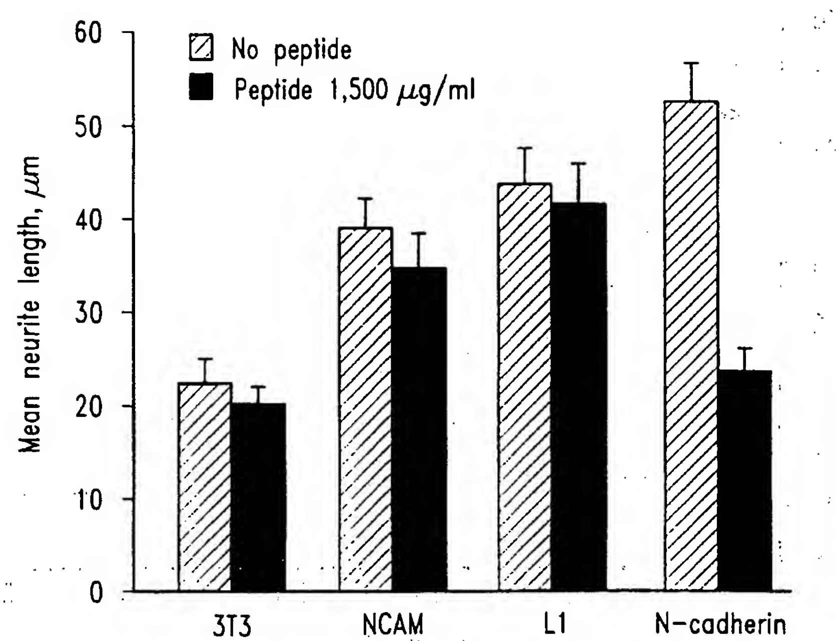

60
50
40
30
20
10
0
Mean neurite length, μm
No peptide
Peptide 1,500 μg/ml
3T3
NCAM
L1
N-cadherin

Fig. 6

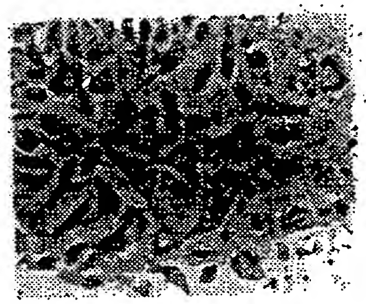


Fig. 9A

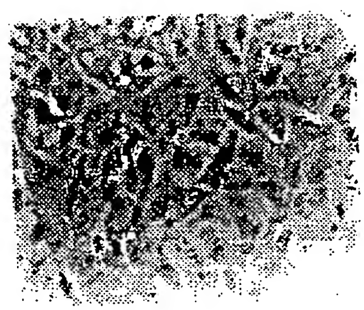


Fig. 9B

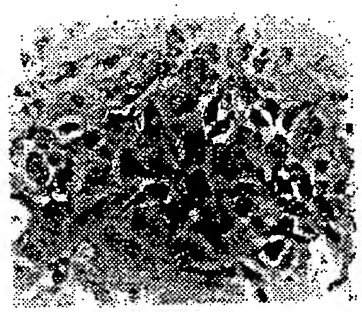


Fig. 9C

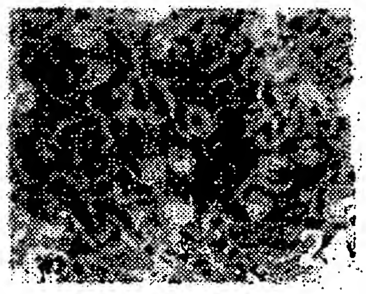


Fig. 10A

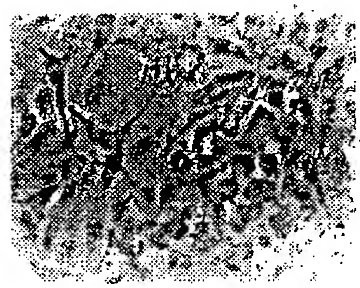


Fig. 10B

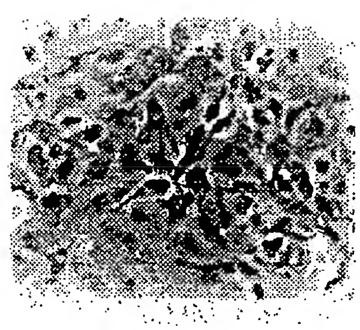


Fig. 10C

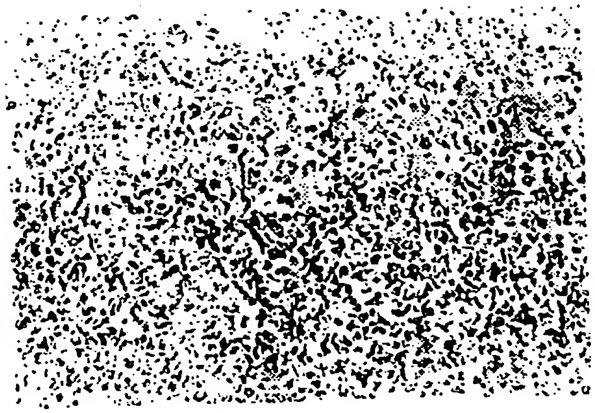


Fig. 11A

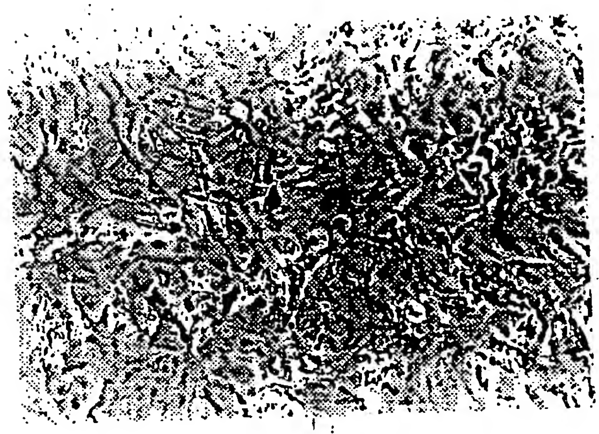


Fig. 11B

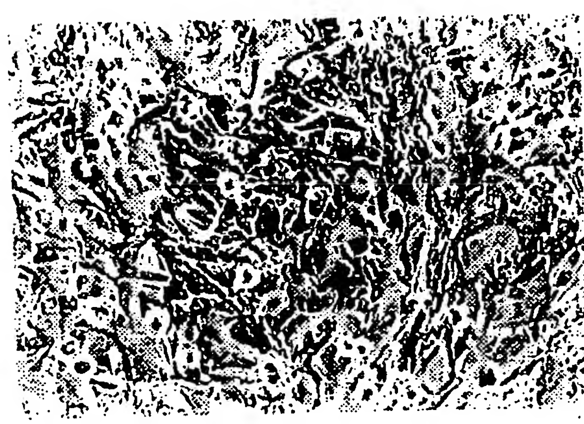


Fig. 11C

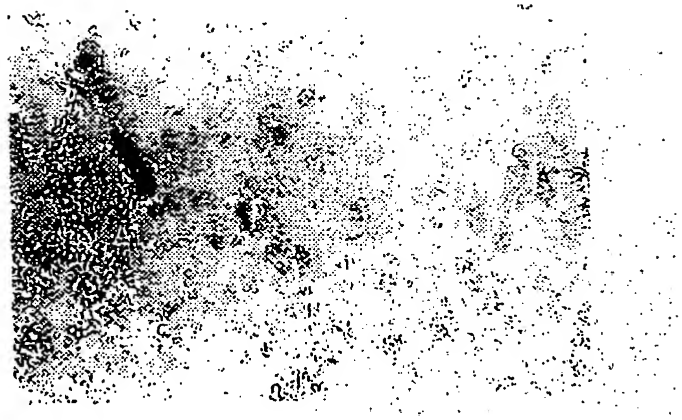


Fig. 11D

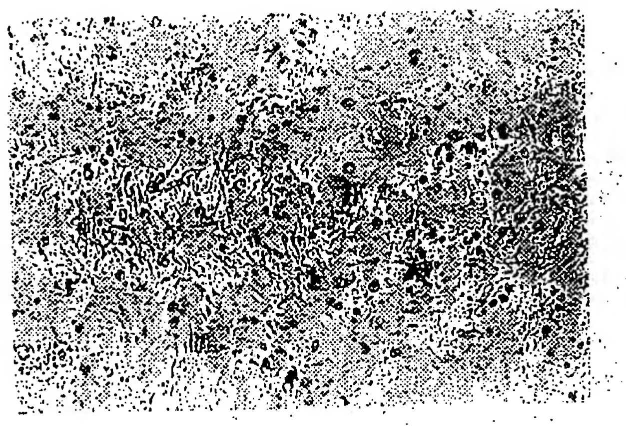


Fig. 11E

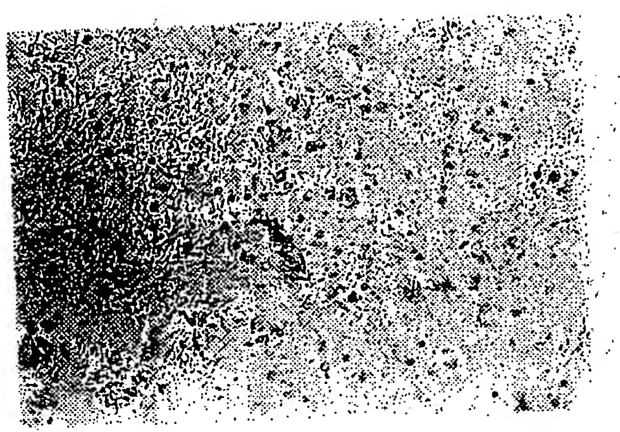


Fig. 11F

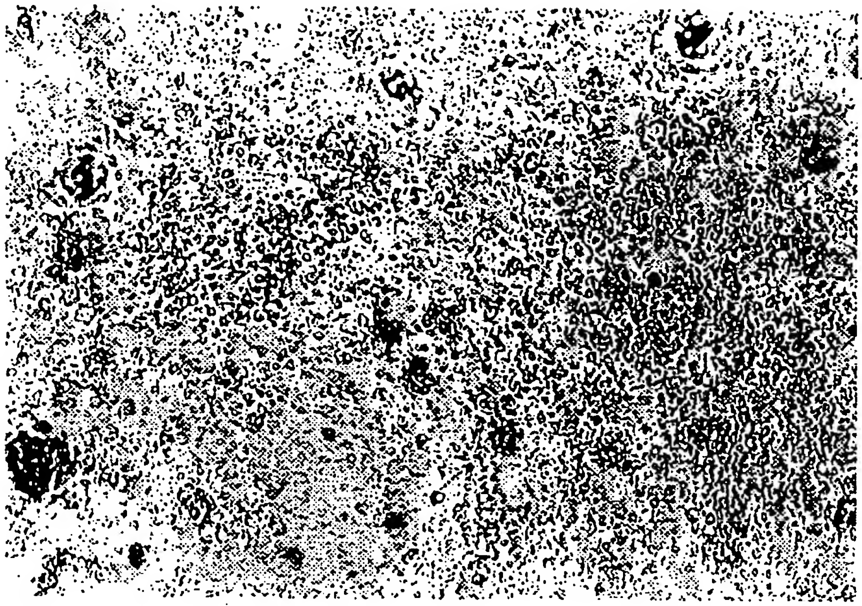


Fig. 12A

Fig. 12B

Untreated

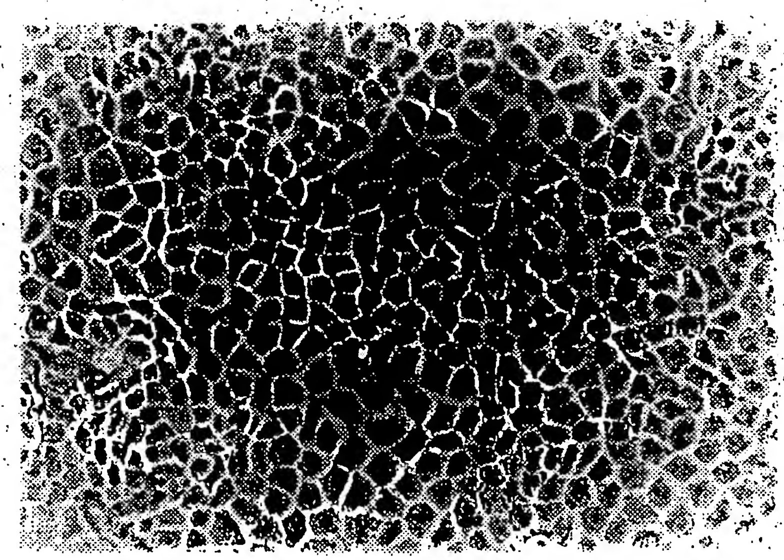


Fig. 13A

HAVS

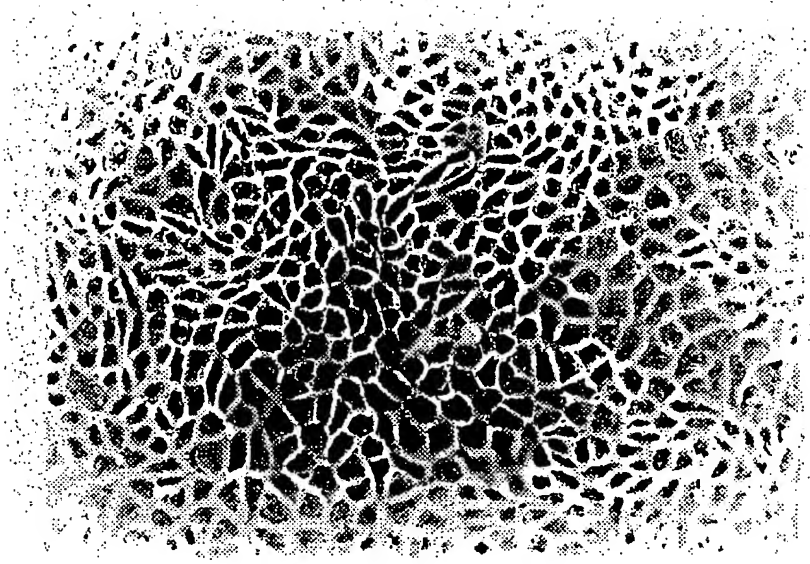


Fig. 13B

HGV

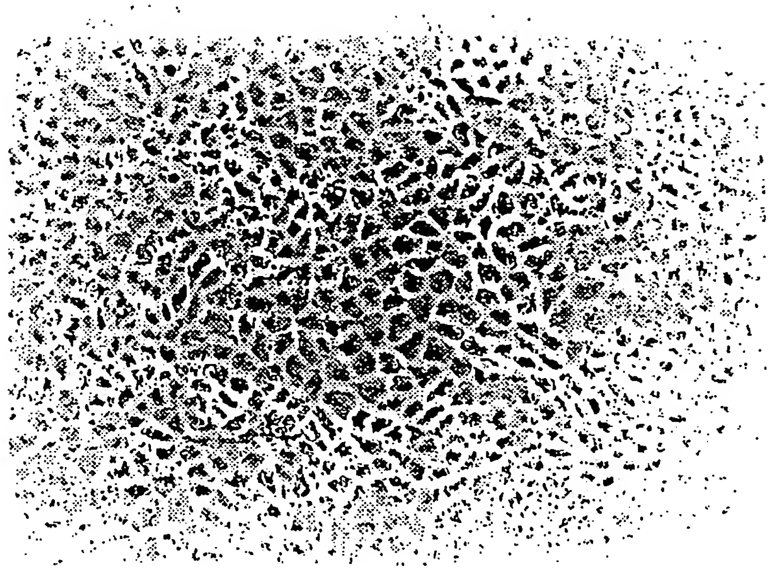


Fig. 13C

HAV

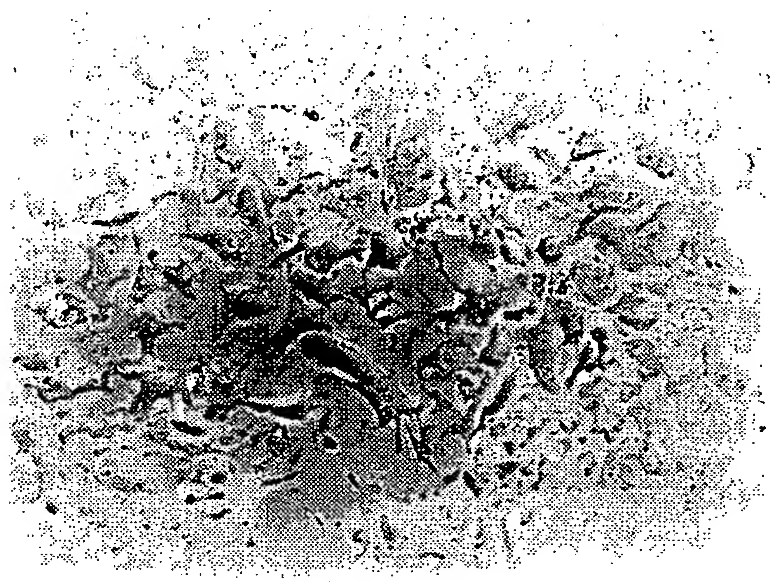


Fig. 13D

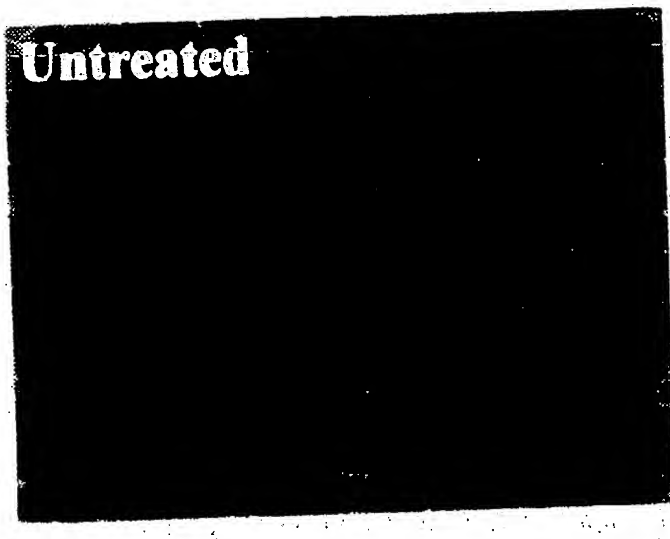


Fig 11A

Fig 11B

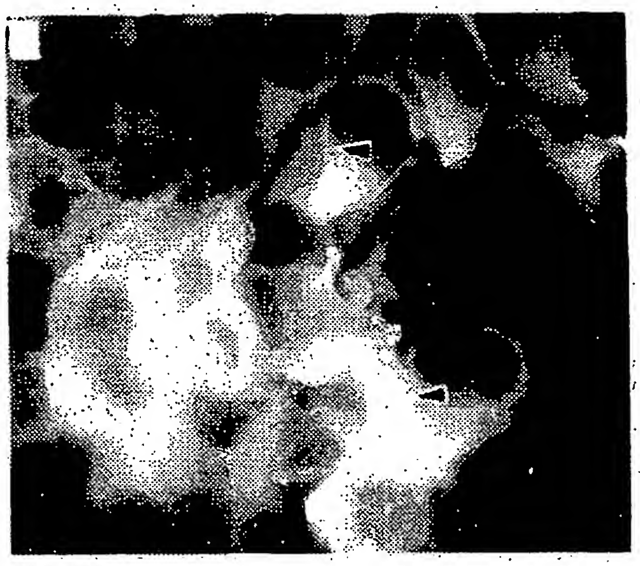


Fig. 16A

Fig. 16B

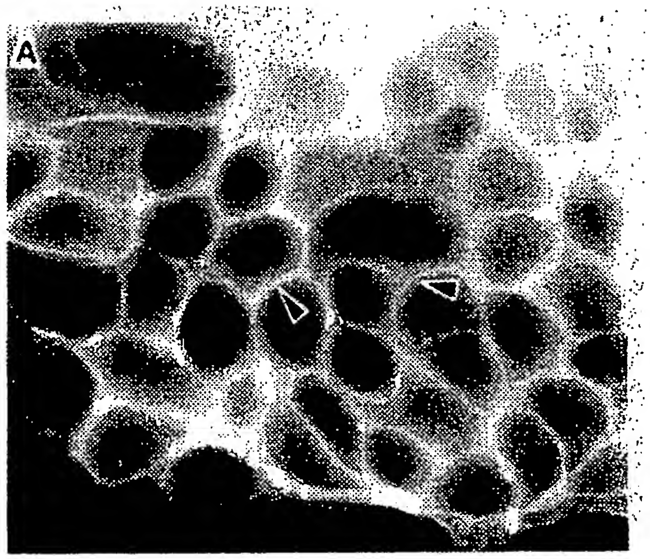


Fig. 17A

Fig. 17B

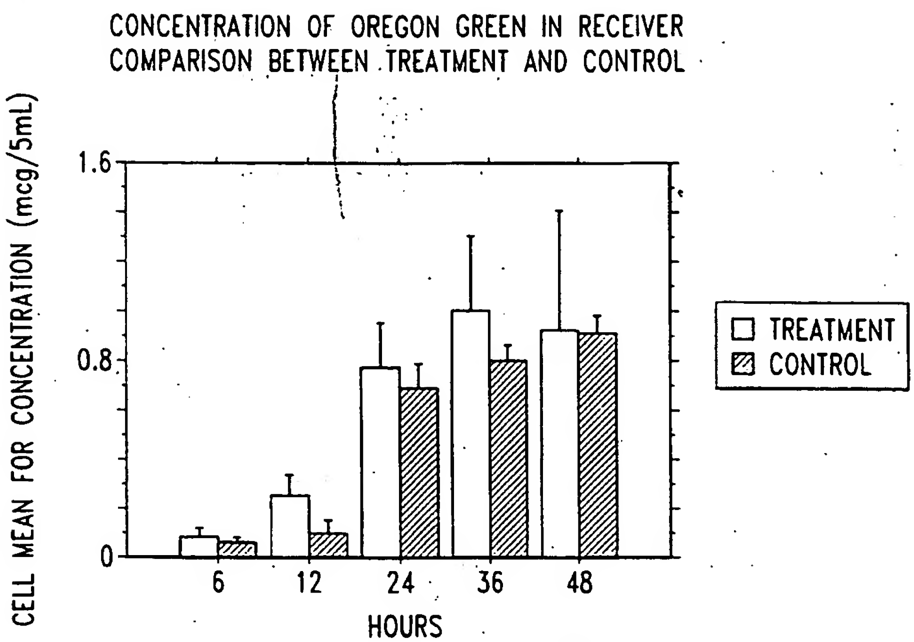

CONCENTRATION OF OREGON GREEN IN RECEIVER
COMPARISON BETWEEN TREATMENT AND CONTROL
CELL MEAN FOR CONCENTRATION (mcg/5mL)
1.6
0.8
0
6
12
24
36
48
HOURS
TREATMENT
CONTROL

Fig. 18

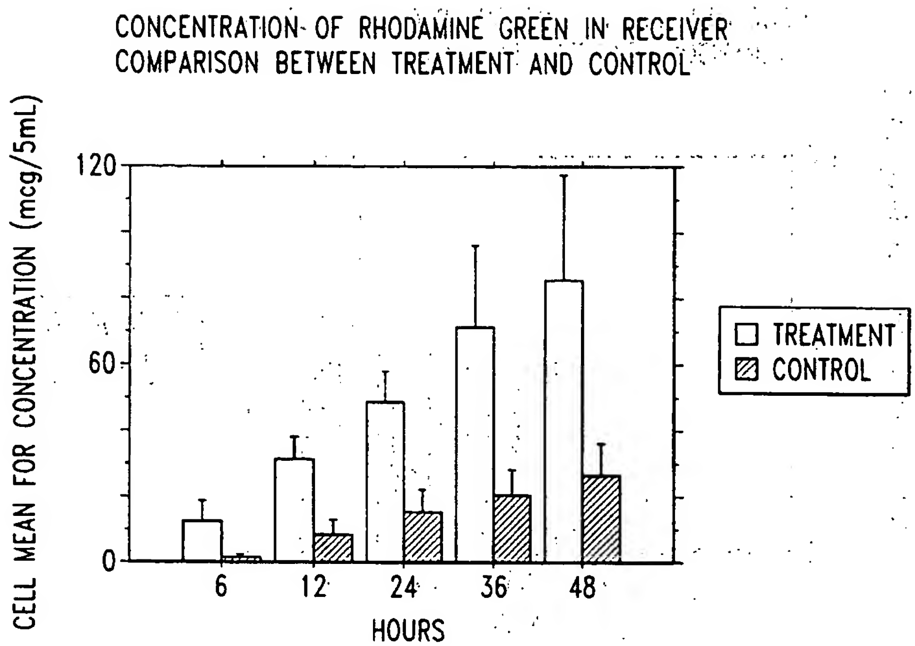
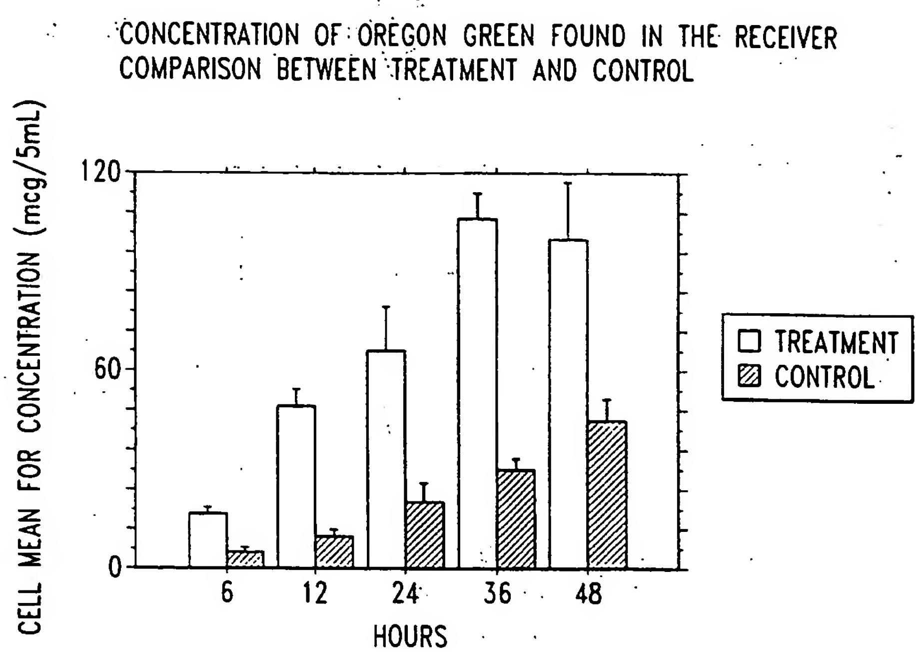

CONCENTRATION OF RHODAMINE GREEN IN RECEIVER
COMPARISON BETWEEN TREATMENT AND CONTROL
CELL MEAN FOR CONCENTRATION (mcg/5mL)
120
60
0
6
12
24
36
48
HOURS
TREATMENT
CONTROL

Fig. 19



CONCENTRATION OF OREGON GREEN FOUND IN THE RECEIVER
COMPARISON BETWEEN TREATMENT AND CONTROL
CELL MEAN FOR CONCENTRATION (mcg/5mL)
120
60
0
6
12
24
36
48
HOURS
TREATMENT
CONTROL

Fig. 20

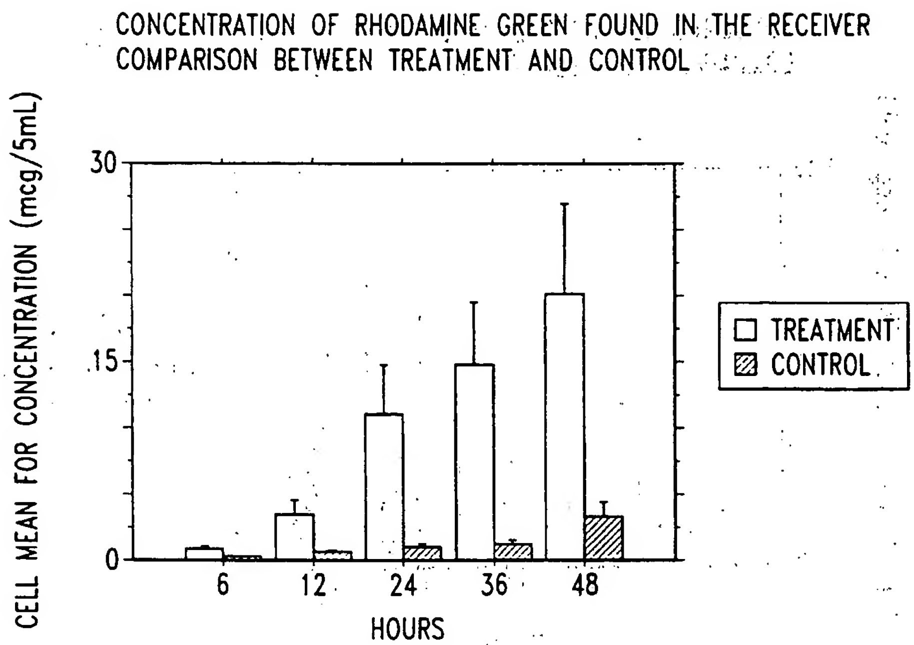

CONCENTRATION OF RHODAMINE GREEN FOUND IN THE RECEIVER
COMPARISON BETWEEN TREATMENT AND CONTROL
CELL MEAN FOR CONCENTRATION (mcg/5mL)
30
.15
0
6
12
24
36
48
HOURS
TREATMENT
CONTROL

Fig. 21

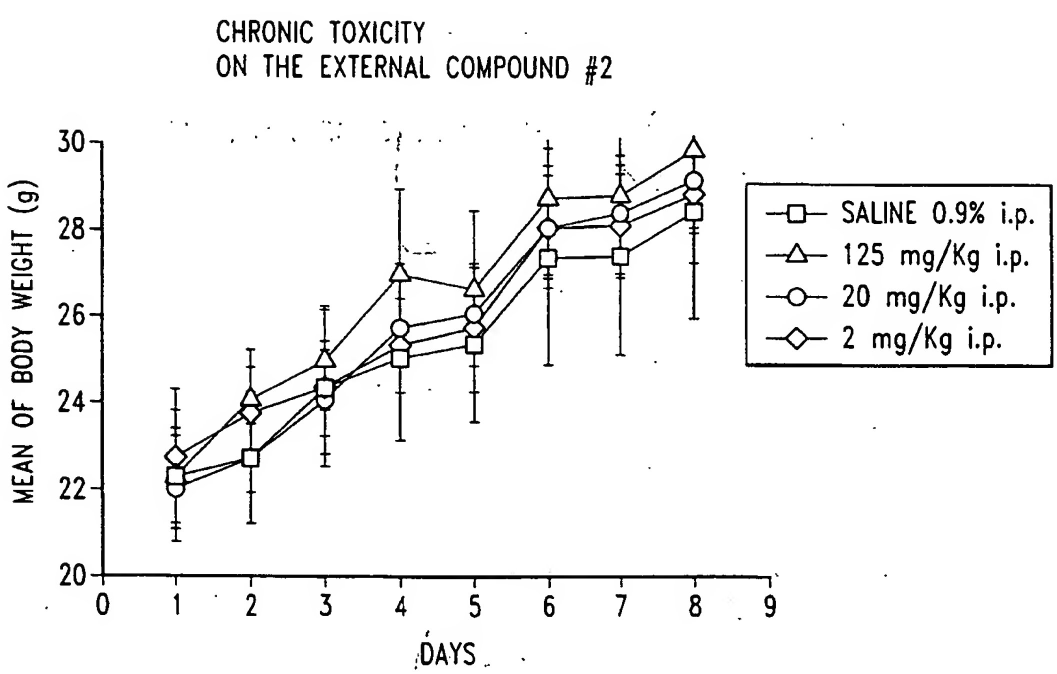

CHRONIC TOXICITY
ON THE EXTERNAL COMPOUND #2
MEAN OF BODY WEIGHT (g)
30
28
26
24
22
20
0
1
2
3
4
5
6
7
8
9
DAYS
SALINE 0.9% i.p.
125 mg/Kg i.p.
20 mg/Kg i.p.
2 mg/Kg i.p.

Fig. 22

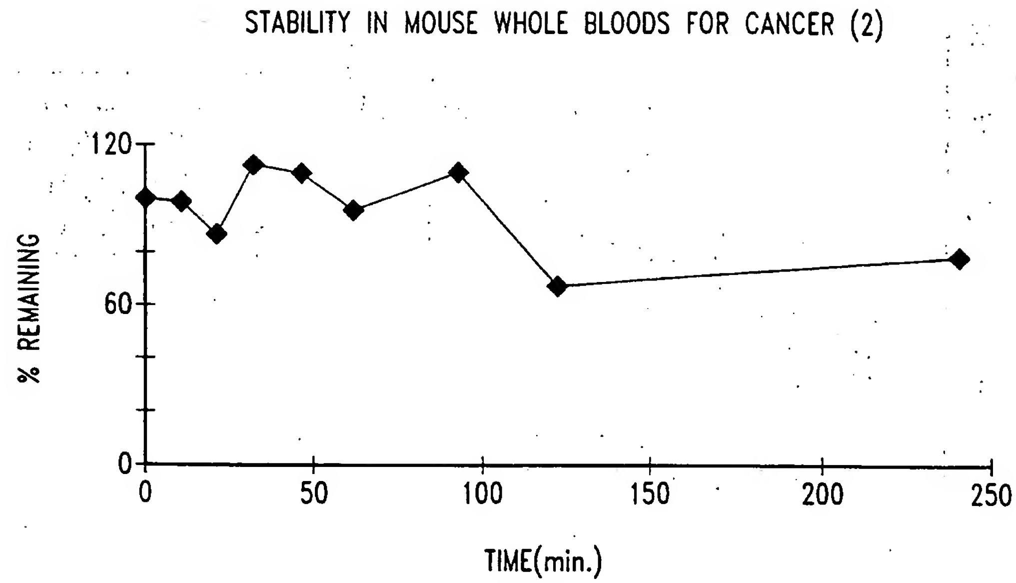

STABILITY IN MOUSE WHOLE BLOODS FOR CANCER (2)
% REMAINING
120
60
0
0
50
100
150
200
250
TIME(min.)

Fig. 23

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: Blaschuk, Orest W.
Gour, Barbara J.

(ii) TITLE OF INVENTION: COMPOUNDS AND METHODS FOR MODULATING SYNAPTIC STABILITY

(iii) NUMBER OF SEQUENCES: 63

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: SEED and BERRY LLP
(B) STREET: 6300 Columbia Center, 701 Fifth Avenue
(C) CITY: Seattle
(D) STATE: Washington
(E) COUNTRY: USA
(F) ZIP: 98104

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 23-DEC-1998
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Maki, Daivd J.
(B) REGISTRATION NUMBER: 31,392
(C) REFERENCE/DOCKET NUMBER: 100086.40101PC

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (206) 622-4900

(B) TELEFAX: (206) 682-6031

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 108 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Asp Trp Val Ile Pro Pro Ile Asn Leu Pro Glu Asn Ser Arg Gly Pro
1               5                   10                  15

Phe Pro Gln Glu Leu Val Arg Ile Arg Ser Asp Arg Asp Lys Asn Leu
            20                  25                  30

Ser Leu Arg Tyr Ser Val Thr Gly Pro Gly Ala Asp Gln Pro Pro Thr
        35                  40                  45

Gly Ile Phe Ile Leu Asn Pro Ile Ser Gly Gln Leu Ser Val Thr Lys
    50                  55                  60

Pro Leu Asp Arg Glu Gln Ile Ala Arg Phe His Leu Arg Ala His Ala
65                  70                  75                  80

Val Asp Ile Asn Gly Asn Gln Val Glu Asn Pro Ile Asp Ile Val Ile
```

85 90 95

Asn Val Ile Asp Met Asn Asp Asn Arg Pro Glu Phe
100 105

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 108 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asp Trp Val Ile Pro Pro Ile Asn Leu Pro Glu Asn Ser Arg Gly Pro
1 5 10 15

Phe Pro Gln Glu Leu Val Arg Ile Arg Ser Asp Arg Asp Lys Asn Leu
20 25 30

Ser Leu Arg Tyr Ser Val Thr Gly Pro Gly Ala Asp Gln Pro Pro Thr
35 40 45

Gly Ile Phe Ile Ile Asn Pro Ile Ser Gly Gln Leu Ser Val Thr Lys
50 55 60

Pro Leu Asp Arg Glu Leu Ile Ala Arg Phe His Leu Arg Ala His Ala
65 70 75 80

Val Asp Ile Asn Gly Asn Gln Val Glu Asn Pro Ile Asp Ile Val Ile
85 90 95

Asn Val Ile Asp Met Asn Asp Asn Arg Pro Glu Phe
100 105

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 108 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Asp Trp Val Ile Pro Pro Ile Asn Leu Pro Glu Asn Ser Arg Gly Pro
1 5 10 15

Phe Pro Gln Glu Leu Val Arg Ile Arg Ser Asp Arg Asp Lys Asn Leu
20 25 30

Ser Leu Arg Tyr Ser Val Thr Gly Pro Gly Ala Asp Gln Pro Pro Thr
35 40 45

Gly Ile Phe Ile Ile Asn Pro Ile Ser Gly Gln Leu Ser Val Thr Lys
50 55 60

Pro Leu Asp Arg Glu Leu Ile Ala Arg Phe His Leu Arg Ala His Ala
65 70 75 80

Val Asp Ile Asn Gly Asn Gln Val Glu Asn Pro Ile Asp Ile Val Ile
85 90 95

Asn Val Ile Asp Met Asn Asp Asn Arg Pro Glu Phe
100 105

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 108 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Asp Trp Val Val Ala Pro Ile Ser Val Pro Glu Asn Gly Lys Gly Pro
1 5 10 15

Phe Pro Gln Arg Leu Asn Gln Leu Lys Ser Asn Lys Asp Arg Asp Thr
20 25 30

Lys Ile Phe Tyr Ser Ile Thr Gly Pro Gly Ala Asp Ser Pro Pro Glu
35 40 45

Gly Val Phe Ala Val Glu Lys Glu Thr Gly Trp Leu Leu Leu Asn Lys
50 55 60

Pro Leu Asp Arg Glu Glu Ile Ala Lys Tyr Glu Leu Phe Gly His Ala
65 70 75 80

Val Ser Glu Asn Gly Ala Ser Val Glu Asp Pro Met Asn Ile Ser Ile
85 90 95

Ile Val Thr Asp Gln Asn Asp His Lys Pro Lys Phe

100 105

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 108 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Glu Trp Val Met Pro Pro Ile Phe Val Pro Glu Asn Gly Lys Gly Pro
1 5 10 15

Phe Pro Gln Arg Leu Asn Gln Leu Lys Ser Asn Lys Asp Arg Gly Thr
20 25 30

Lys Ile Phe Tyr Ser Ile Thr Gly Pro Gly Ala Asp Ser Pro Pro Glu
35 40 45

Gly Val Phe Thr Ile Glu Lys Glu Ser Gly Trp Leu Leu Leu His Met
50 55 60

Pro Leu Asp Arg Glu Lys Ile Val Lys Tyr Glu Leu Tyr Gly His Ala
65 70 75 80

Val Ser Glu Asn Gly Ala Ser Val Glu Glu Pro Met Asn Ile Ser Ile
85 90 95

Ile Val Thr Asp Gln Asn Asp Asn Lys Pro Lys Phe
100 105

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 108 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Asp Trp Val Ile Pro Pro Ile Ser Cys Pro Glu Asn Glu Lys Gly Pro
1               5                   10                  15

Phe Pro Lys Asn Leu Val Gln Ile Lys Ser Asn Lys Asp Lys Glu Gly
            20                  25                  30

Lys Val Phe Tyr Ser Ile Thr Gly Gln Gly Ala Asp Thr Pro Pro Val
        35                  40                  45

Gly Val Phe Ile Ile Glu Arg Glu Thr Gly Trp Leu Lys Val Thr Glu
    50                  55                  60

Pro Leu Asp Arg Glu Arg Ile Ala Thr Tyr Thr Leu Phe Ser His Ala
65                  70                  75                  80

Val Ser Ser Asn Gly Asn Ala Val Glu Asp Pro Met Glu Ile Leu Ile
                85                  90                  95

Thr Val Thr Asp Gln Asn Asp Asn Lys Pro Glu Phe
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 108 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Asp Trp Val Ile Pro Pro Ile Ser Cys Pro Glu Asn Glu Lys Gly Glu
1               5                   10                  15

Phe Pro Lys Asn Leu Val Gln Ile Lys Ser Asn Arg Asp Lys Glu Thr
            20                  25                  30

Lys Val Phe Tyr Ser Ile Thr Gly Gln Gly Ala Asp Lys Pro Pro Val
        35                  40                  45

Gly Val Phe Ile Ile Glu Arg Glu Thr Gly Trp Leu Lys Val Thr Gln
    50                  55                  60

Pro Leu Asp Arg Glu Ala Ile Ala Lys Tyr Ile Leu Tyr Ser His Ala
65                  70                  75                  80

Val Ser Ser Asn Gly Glu Ala Val Glu Asp Pro Met Glu Ile Val Ile
                85                  90                  95

Thr Val Thr Asp Gln Asn Asp Asn Arg Pro Glu Phe
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Cys His Ala Val Cys
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Cys His Gly Val Cys
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Cys Ala His Ala Val Asp Ile Cys
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Cys Ala His Gly Val Asp Ile Cys
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Cys Ser His Ala Val Cys
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Cys Ser His Gly Val Cys
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Cys His Ala Val Ser Cys
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Cys His Gly Val Ser Cys
1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Cys Ala His Ala Val Asp Cys
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Cys Ala His Gly Val Asp Cys
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Cys Ser His Ala Val Ser Ser Cys
1 5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Cys Ser His Gly Val Ser Ser Cys
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Lys His Ala Val Asp
1   5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Lys His Gly Val Asp
1   5

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Cys Ala His Ala Val Asp Ile Pro
1 5

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Cys Ala His Gly Val Asp Ile Pro
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

(D) OTHER INFORMATION: /product= "Dbu"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Xaa His Ala Val Ser Gly
1   5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

(D) OTHER INFORMATION: /product= "Orn"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Xaa His Ala Val Ser
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Leu Ala His Ala Val Asp Ile
1 5

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Leu Ala His Gly Val Asp Ile
1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION: /product= "OTHER"
/note= "Residue is beta,beta-dimethyl cysteine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Cys His Ala Val Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 2

(D) OTHER INFORMATION: /product= "OTHER"

/note= "Residue is beta,beta-tetramethylene cysteine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Ile Xaa Tyr Ser His Ala Val Ser Cys
1 5

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 2

(D) OTHER INFORMATION: /product= "OTHER"

/note= "Residue is beta,beta-pentamethylene cysteine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ile Xaa Tyr Ser His Ala Val Ser Ser Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /product= "OTHER"
/note= "Residue is beta-mercatopropionic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Xaa Tyr Ser His Ala Val Ser Ser Cys
1 5

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

(D) OTHER INFORMATION: /product= "OTHER"

/note= "Residue is
beta,beta-pentamethylene-beta-mercaptopropionic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Xaa Tyr Ser His Ala Val Ser Ser Cys
1 5

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Cys Asp Gly Tyr Pro Lys Asp Cys Lys Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Cys Asp Gly Tyr Pro Lys Asp Cys Lys Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Cys Gly Asn Leu Ser Thr Cys Met Leu Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Cys Gly Asn Leu Ser Thr Cys Met Leu Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Cys Tyr Ile Gln Asn Cys Pro Leu Gly
1 5

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Cys Tyr Ile Gln Asn Cys Pro Leu Gly
1 5

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Trp Gly Gly Trp
1

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Leu Asp Arg Glu
1

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Asp Xaa Asn Asp Asn
1 5

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Xaa Asp Xaa Glu
1

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Asp Val Asn Glu
1

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Ala His Ala Val Asp Ile
1 5

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Ser His Ala Val Ser Ser
1 5

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Lys Ser His Ala Val Ser Ser Asp
1 5

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Tyr Ile Gly Ser Arg
1 5

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Cys His Ala Val Asp Cys
1 5

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Cys Ala His Ala Val Cys
1   5

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Cys Arg Ala His Ala Val Asp Cys

1 5

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Cys Leu Arg Ala His Ala Val Cys
1 5

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Cys Leu Arg Ala His Ala Val Asp Cys

1 5

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Cys Ser His Ala Val Ser Cys
1 5

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Cys His Ala Val Ser Ser Cys

1 5

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Asp His Ala Val Lys

1 5

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Lys His Ala Val Glu

1 5

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Ser His Ala Val Asp Ser Ser
1 5

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Leu Tyr His Tyr

1

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 48 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Gly Val Asn Pro Thr Ala Gln Ser Ser Gly Ser Leu Tyr Gly Ser Gln
1 5 10 15

Ile Tyr Ala Leu Cys Asn Gln Phe Tyr Thr Pro Ala Ala Thr Gly Leu
20 25 30

Tyr Val Asp Gln Tyr Leu Tyr His Tyr Cys Val Val Asp Pro Gln Glu
35 40 45

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Gln Tyr Leu Tyr His Tyr Cys Val Val Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Phe His Leu Arg Ala His Ala Val Asp Ile Asn Gly Asn Gln Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Lys Tyr Ser Phe Asn Tyr Asp Gly Ser Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Ile Trp Lys His Lys Gly Arg Asp Val Ile Leu Lys Lys Asp Val Arg
1 5 10 15

Phe

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 98/01207

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/705 C07K16/28 C07K7/64 A61K38/17 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 02452 A (UNIV MCGILL) 22 January 1998 see the whole document ---	1-33
P,X	WO 98 45319 A (UNIV MCGILL ;GOUR BARBARA J (CA); BLASCHUK OREST W (CA)) 15 October 1998 see the whole document ---	1-33
A	P.W. BEESLEY ET AL: "The post-synaptic demsity: putative involvment in synapse stabilization via cadherins and covalent modification by ubiquitination" BIOCHEMICAL SOCIETY TRANSACTIONS, vol. 23, no. 1, February 1995, pages 59-64, XP002102493 see page 59, column 2, paragraph 1 ---	

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
11 May 1999	27/05/1999

Name and mailing address of the ISA	Authorized officer
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1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 04745 A (ATHENA NEUROSCIENCES INC) 18 April 1991	
A	WO 94 11401 A (UNIV YALE) 26 May 1994	
A	US 5 646 250 A (SUZUKI SHINTARO) 8 July 1997	
A	LUTZ K L ET AL: "SECONDARY STRUCTURE OF THE HAV PEPTIDE WHICH REGULATES CADHERIN -CADHERIN INTERACTION" JOURNAL OF BIOMOLECULAR STRUCTURE & DYNAMICS, vol. 13, no. 3, 1 December 1995, pages 447-455, XP002050190	
A	BLASCHUK O W ET AL: "IDENTIFICATION OF A CADHERIN CELL ADHESION RECOGNITION SEQUENCE" DEVELOPMENTAL BIOLOGY, vol. 139, no. 1, 1 May 1990, pages 227-229, XP002050189	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 98/01207

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-33
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-33 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 98/01207

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9802452 A	22-01-1998	AU 3332297 A	09-02-1998
WO 9845319 A	15-10-1998	AU 6914798 A	30-10-1998
WO 9104745 A	18-04-1991	CA 2067183 A	28-03-1991
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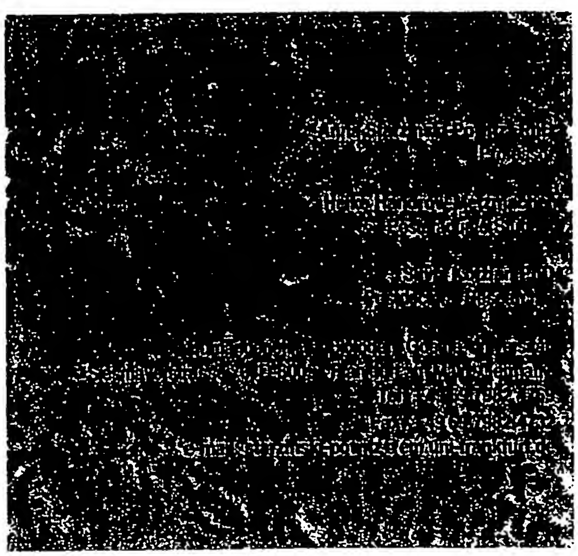
REV 7/02/03

The putative role of cell adhesion molecules in endometriosis: can we learn from tumour metastasis?

Anna Starzinski-Powitz, Heike Handrow-Metzmacher and Silvia Kotzian

Endometriosis, one of the most frequent diseases in gynaecology, is a considerable threat to the physical, psychological and social integrity of women. The etiology and pathogenesis of this important disease, defined as the ectopic location of endometrium-like glandular epithelium and stroma outside the uterine cavity, is poorly understood. Clinical observations and *in vitro* experiments imply that endometriotic cells are invasive and able to metastasize. Analogous to tumour metastasis, it is likely that cell adhesion molecules are central for the invasion and metastasis of endometriotic cells. Investigation of these molecules in endometriosis should increase our understanding of the molecular mechanisms involved in the pathogenesis of this disease.

ENDOMETRIOSIS is an invasive but benign gynaecological disease that is characterized histologically by the presence of endometrial-like tissue (glandular epithelium and stroma) outside the uterine cavity. It is one of the most frequent diseases in gynaecology, affecting 15–20% of women in their reproductive life span[1]. The endometriotic tissue often undergoes cyclic proliferation and breakdown similar to eutopic endometrium, resulting in local inflammatory reactions. These processes cause the cyclical character of endometriosis with dysmenorrhea, dyspareunia, pelvic pain, catamenial hematuria and other symptoms derived from the affected organ (see Box 1). Moreover, up to 50% of infertile patients have this disease[2].



Thus, although not malignant, endometriosis causes significant physical pathology in affected women, as well as causing psychological and social problems. Considering this and the frequency of endometriosis, it is stunning how little research effort has been put into understanding the molecular basis of this important disease.

Currently, it is postulated that the majority of endometriotic lesions are derived from viable eutopic endometrial cells that are transported to the peritoneal cavity by retrograde menstruation, which then adhere to the peritoneal wall, proliferate and form endometriotic lesions (the implantation theory[3]). Retrograde menstruation occurs in almost all women and can be a continuous cellular source for the maintenance of endometriosis[4]. A second possibility is that endometriosis is established, at least in some cases, by sex hormone-dependent transformation of peritoneal cells into Müllerian-type epithelium (the metaplasia theory[5]). The implantation theory is supported by: (1) the observation that viable endometrial cells have the capacity to implant; (2) by the predominant localization of implants in the lower pelvis; and (3) by experiments in which endometrium has been transplanted into nude mice. The metaplasia theory provides an explanation for the

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Box 1. Endometriosis

[illegible]

occurrence of endometriosis in men and in women without retrograde menstruation.

Endometriotic lesions can be located superficially on the peritoneum (peritoneal endometriosis) or in ovaries (ovarian endometriosis). Furthermore, deep, infiltrating lesions are sometimes found in patients, suggesting that variable factors (e.g. peritoneal fluid versus blood factors) might influence the development and progression of the disease (reviewed in Ref. 6). Regardless of the mechanisms of the initiation and progression of endometriotic lesions, clinical and histological parameters define it as a benign but invasive disease[7,8]. Thus, endometriotic cells must be able to penetrate organs of the pelvic cavity, as indicated by deep lesions in the rectovaginal tissue and intestine (see Fig. 1) or more distant organs, such as the lung or lymph nodes, after spreading via the bloodstream or lymphatic system. It is likely that these properties of endometriotic cells contribute to the pathogenesis of endometriosis.

Glossary

Cadherins – A family of Ca^{2+}-dependent transmembrane proteins that mediates cell–cell adhesion. Cadherins play an essential role in the formation of cellular junctions and contribute to tissue development.

Catamenial hematuria – Blood in the urine at the time of menstruation.

Collagen-gel-invasion assay – An *in vitro* experiment that tests the invasive capacity of cells. Cells are cultivated on a collagen gel – only cells with invasive potential are able to invade it.

Dysmenorrhea – Period pain.

Dyspareunia – A collective name for pain during sexual intercourse, particularly in women.

Focal contacts – Cell adhesion sites with the extracellular matrix that are mediated by proteins of the integrin family, which are connected to the actin filaments.

Hemidesmosomes – Structures that connect cells to the extracellular matrix. Intracellularly, the hemidesmosomal transmembrane proteins complex with intermediate filaments.

Integrins – A family of transmembrane proteins that mediates adhesion mainly between cells and components of the extracellular matrix, such as fibronectin, laminin, collagen or tenascin.

Invasive – Describes in this review the invasion capacity of cells *in vitro*, for example in the collagen-gel-invasion assay.

Metastatic – Describes in this review the capacity of cells to spread *in situ*.

Retrograde menstruation – The retrograde efflux of endometrial cells through the oviducts into the peritoneal cavity during menstruation.

Zonula adherens – A region of defined cadherin-mediated cell–cell contacts that forms a belt around the cell and permits tight cell cohesion.

Figure 1. Histological sections of (a) healthy eutopic endometrium and (b,c) of two deep intestinal endometriotic lesions. The endometriotic lesions contain glandular epithelium (arrowheads), which is very similar to that of normal eutopic endometrium. The endometriotic lesions in (c) are filled with hemosiderin formed by the breakdown of hemoglobin.